Figure 1: Examples of Nuclease Stable Ribozyme Motifs

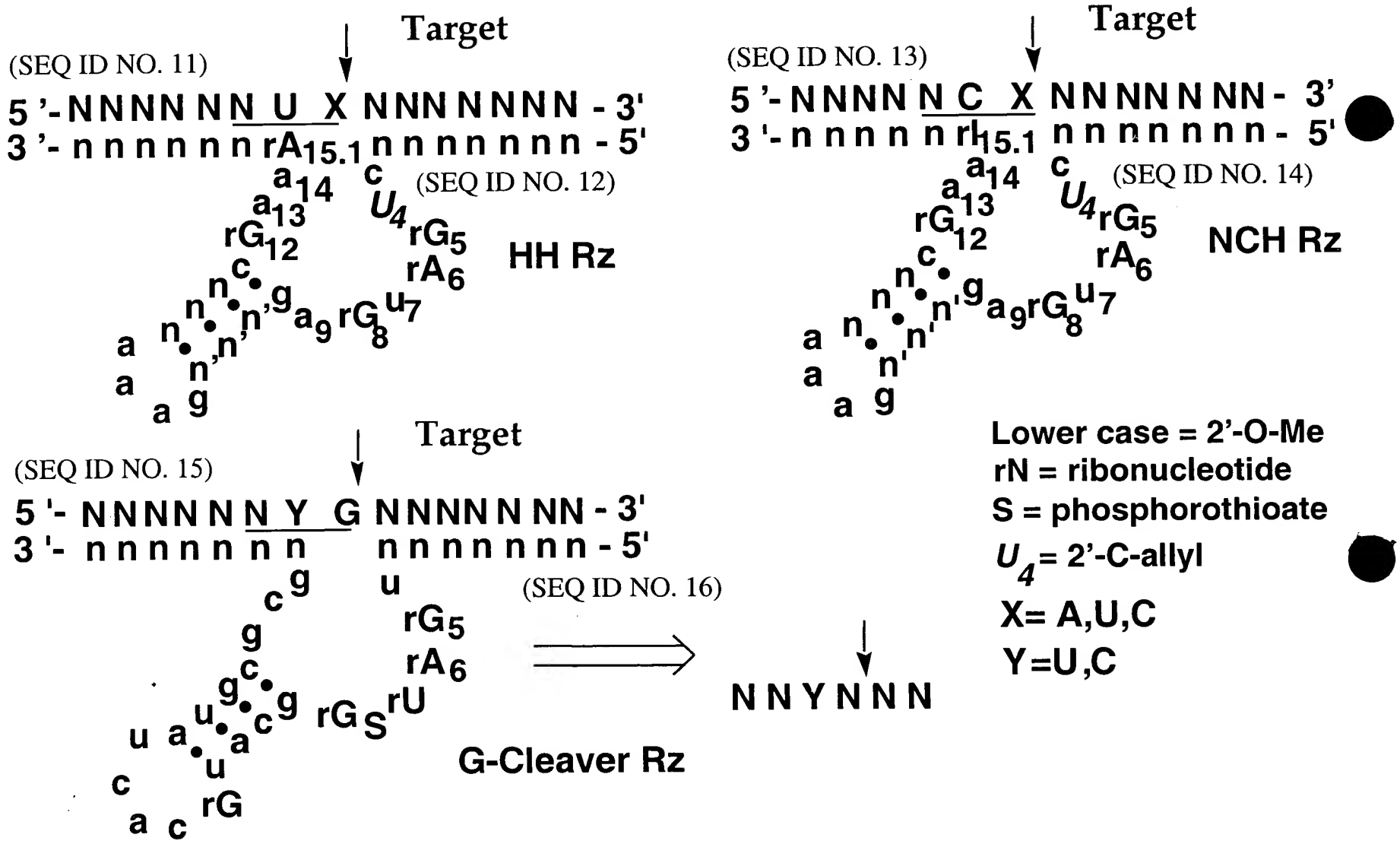


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

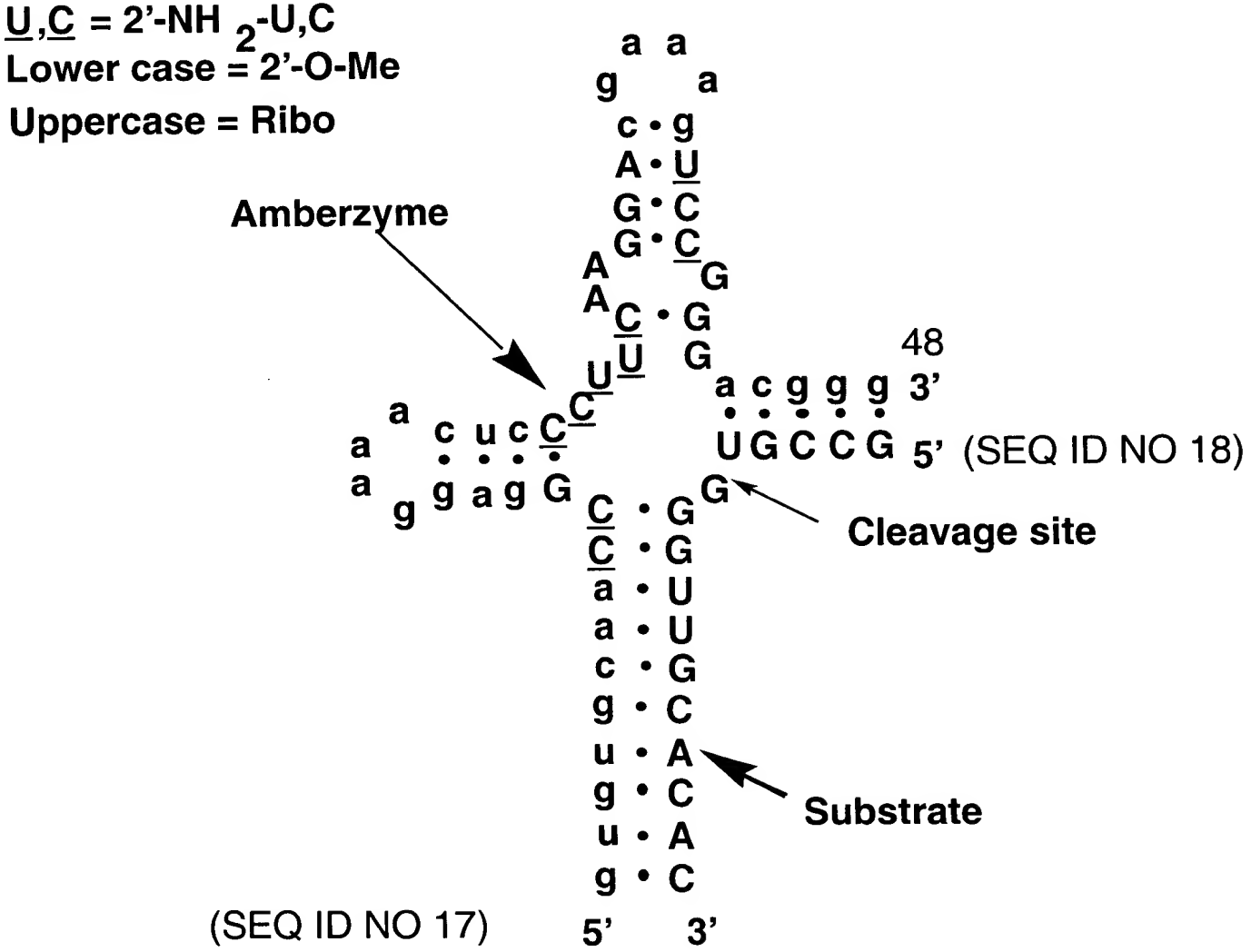
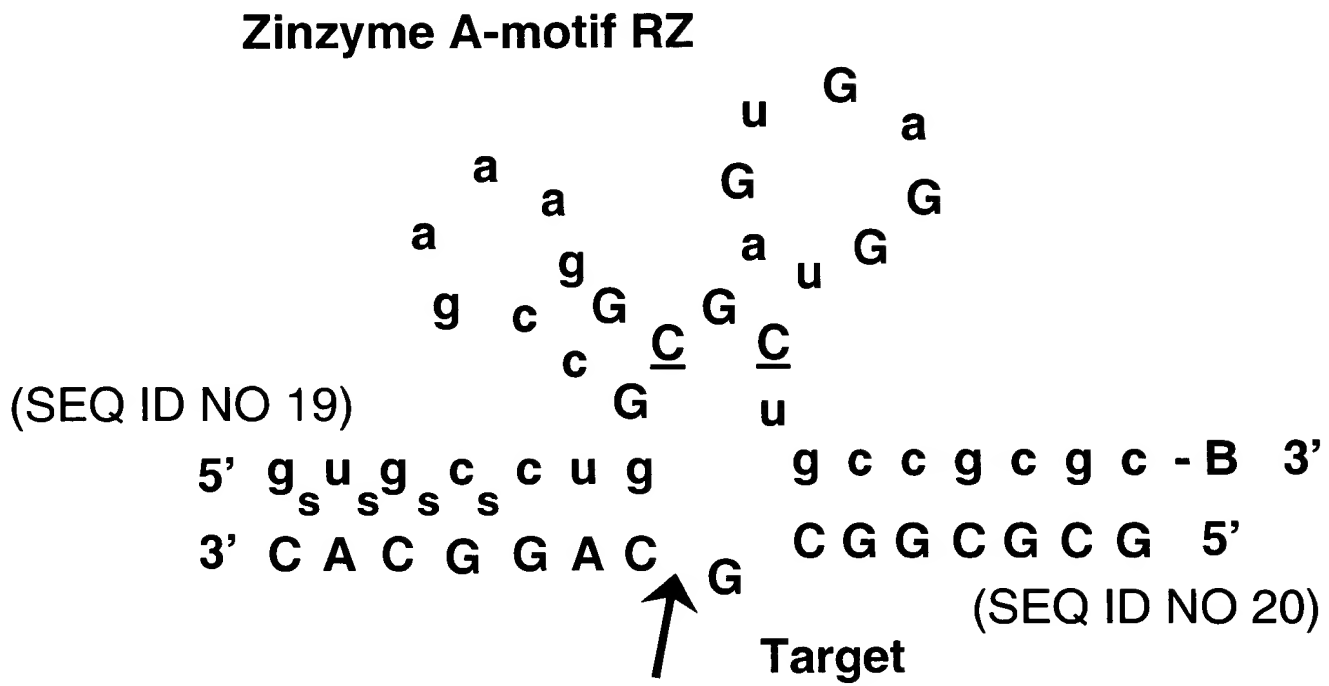


Figure 3: Stabilized Zinzyme Ribozyme Motif



Legend

Uppercase indicates natural ribo residues

C indicates 2'- d-NH_2-C

Lowercase: 2'-O-Me

Subscript $_s$ indicates phosphothioate linkage

B: 3'- 3' abasic moiety

Figure 4: DNAzyme Motif

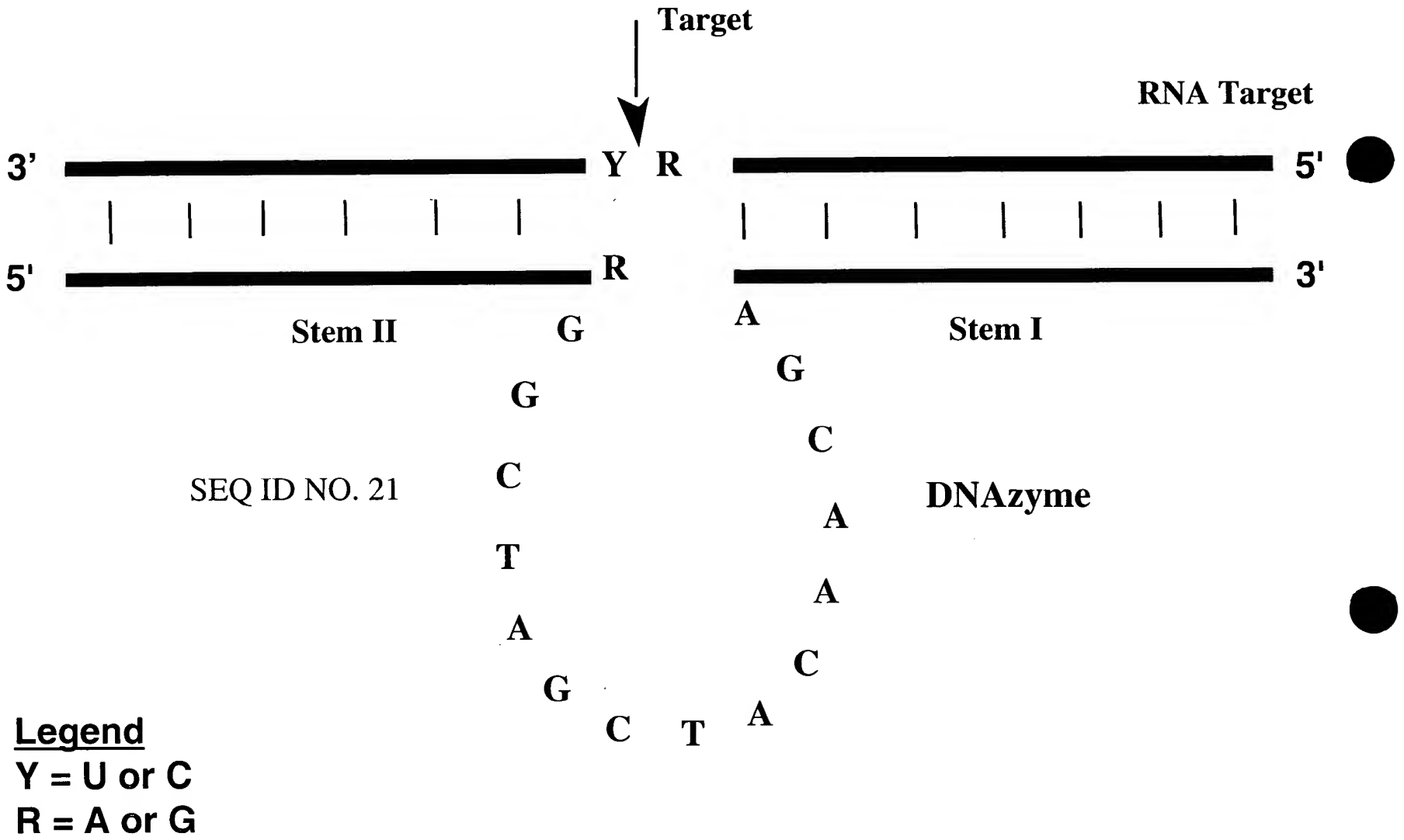


Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence

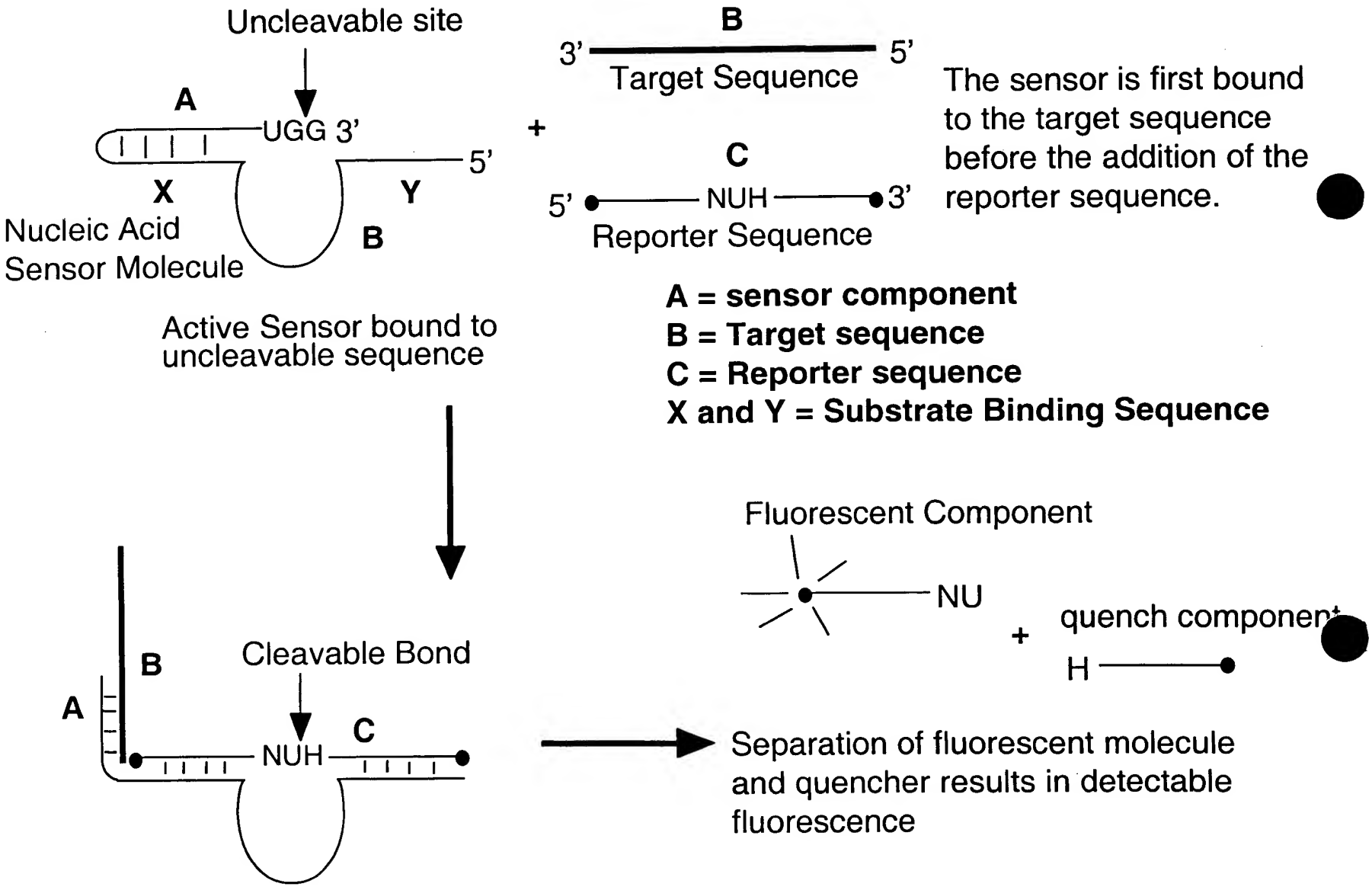


Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule

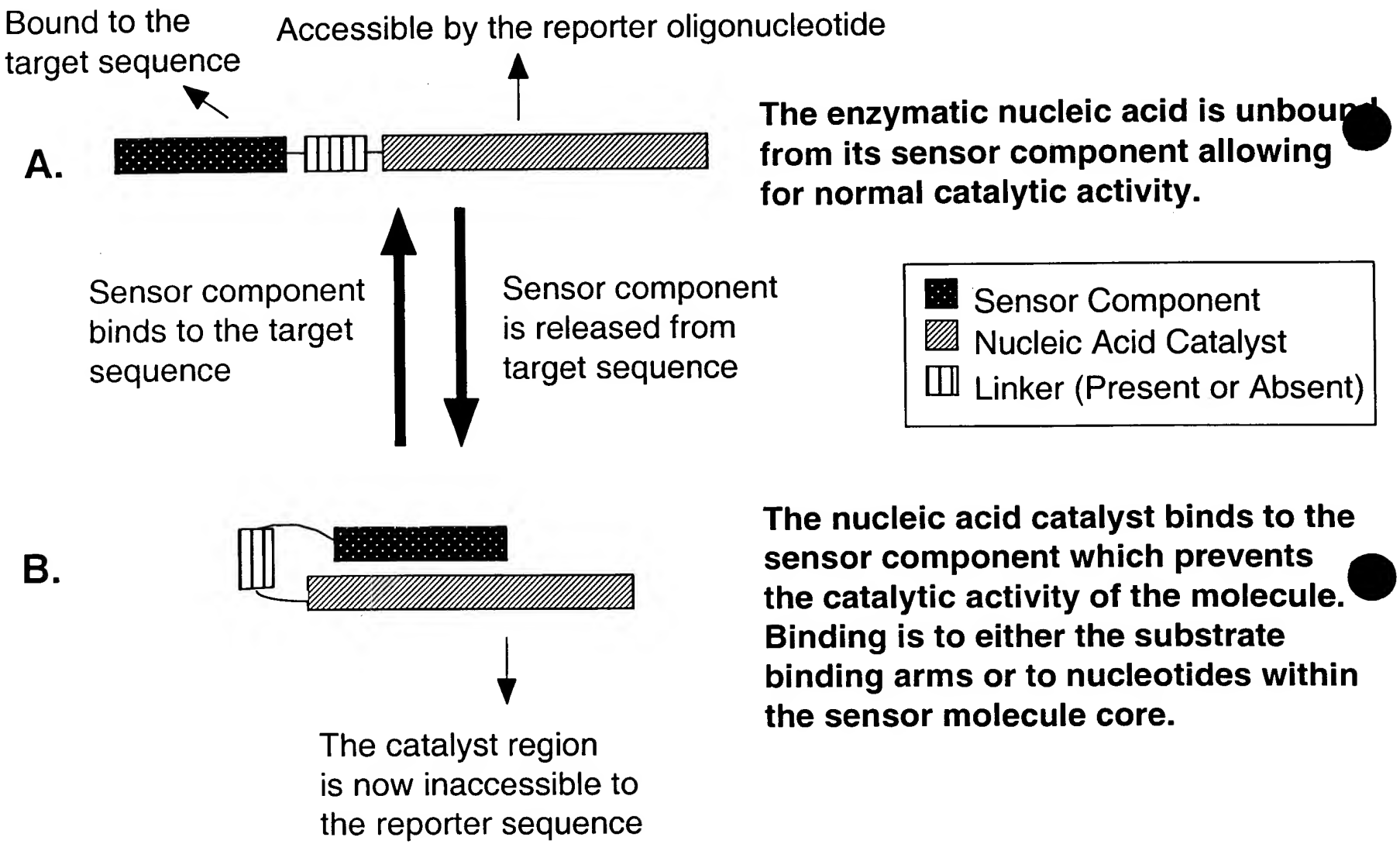


Figure 7a. Examples of Diagnostic Effector Molecules

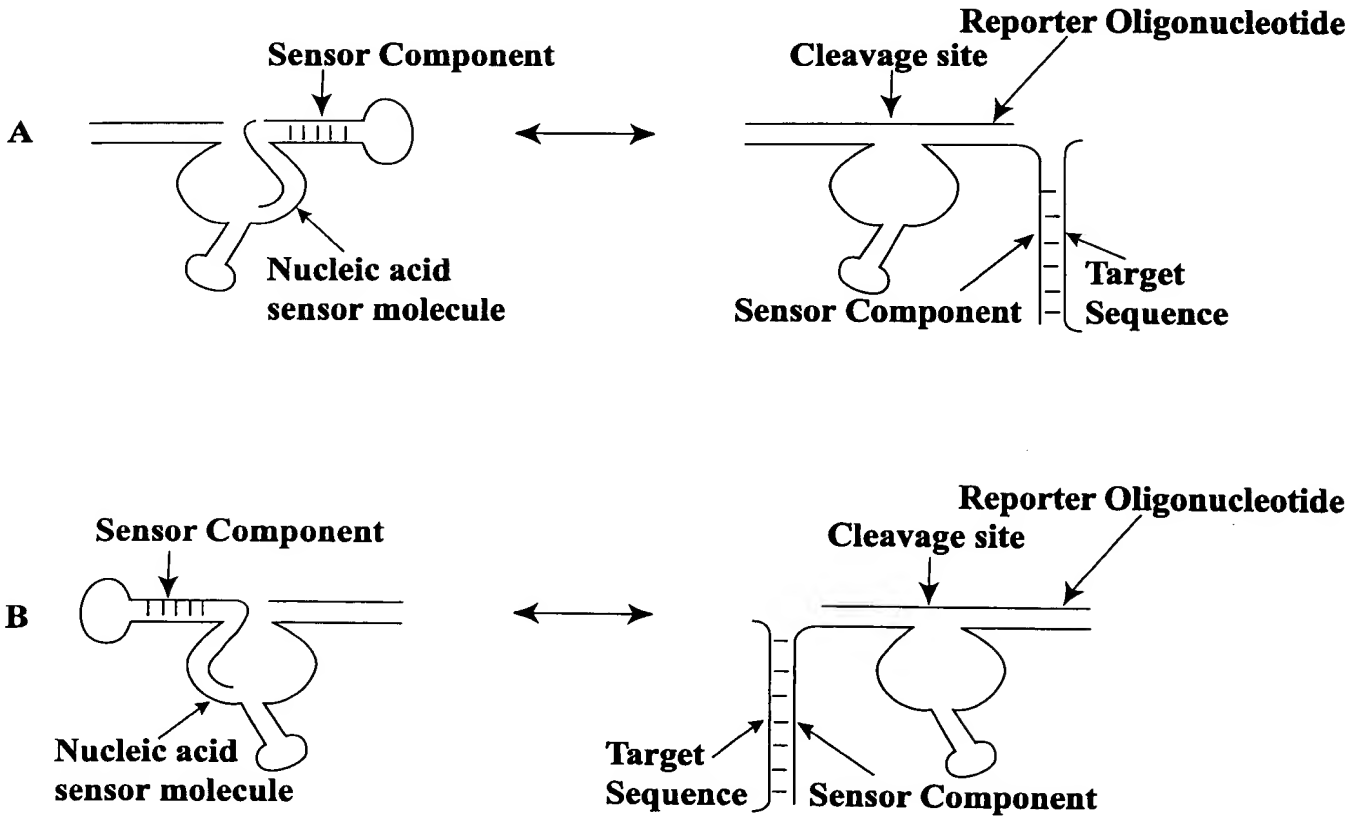


Figure 7b. Examples of Diagnostic Effector Molecules

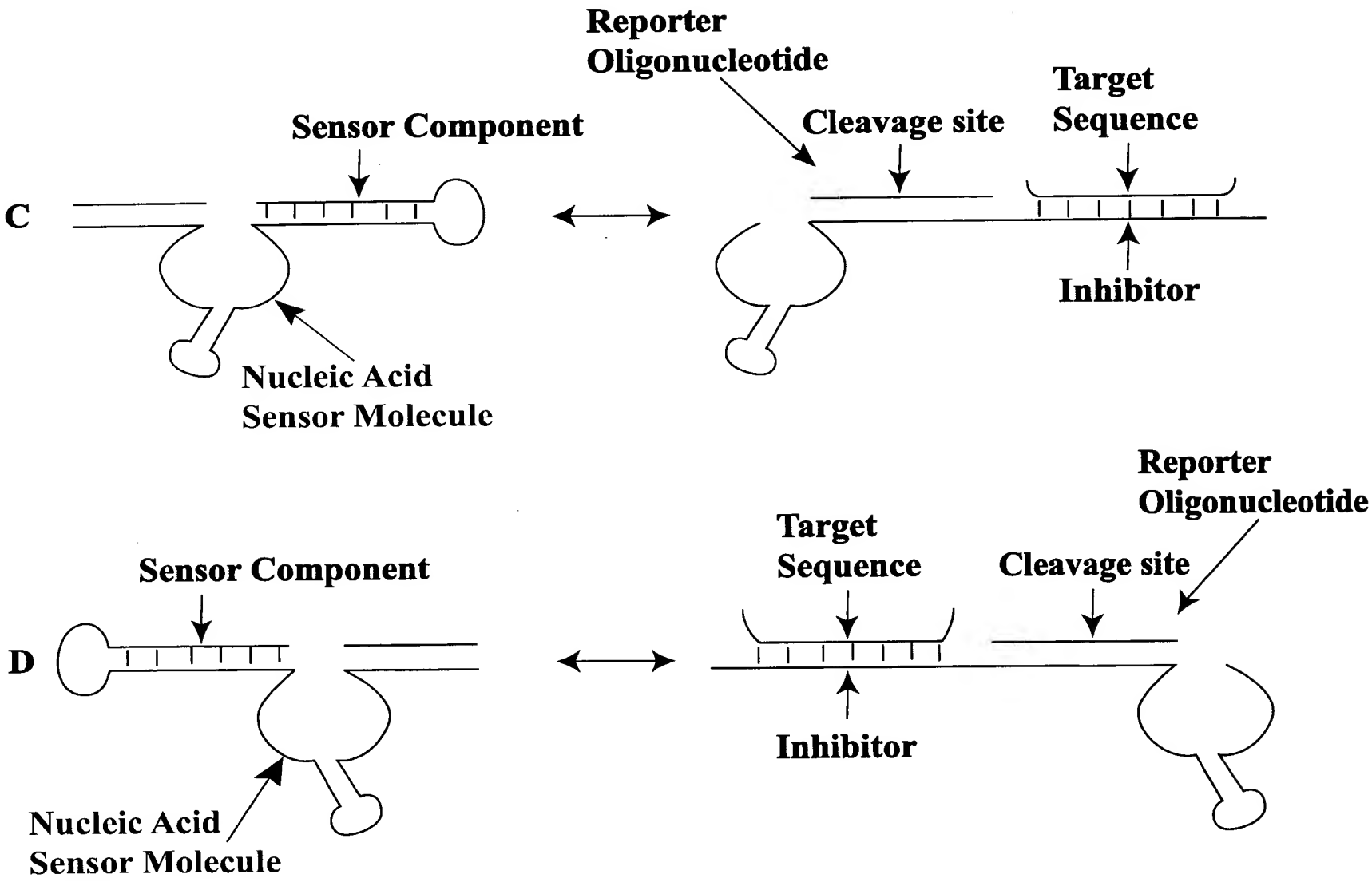


Figure 8a. Examples of Diagnostic Effector Molecules

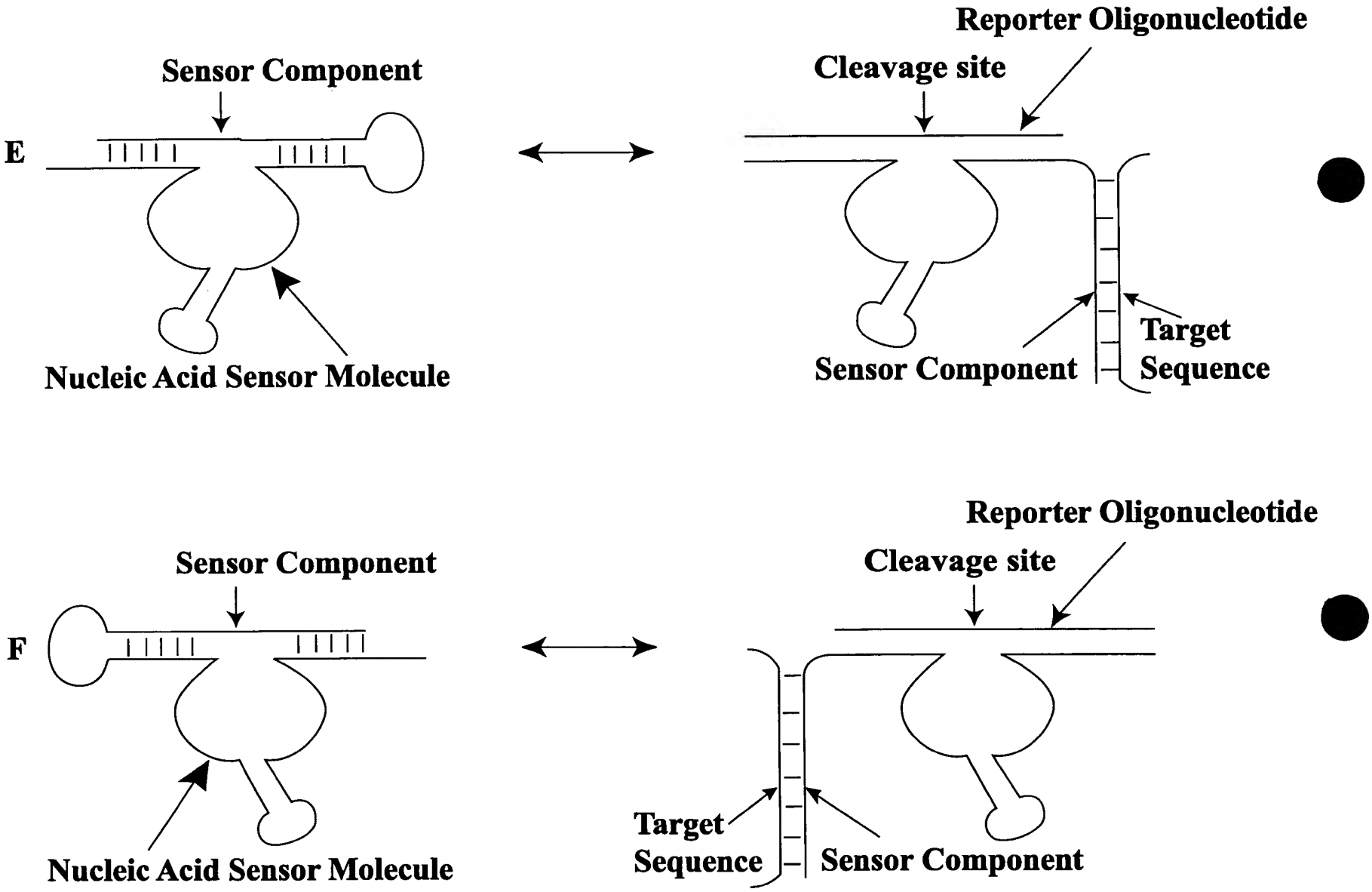


Figure 8b. Examples of Diagnostic Effector Molecules

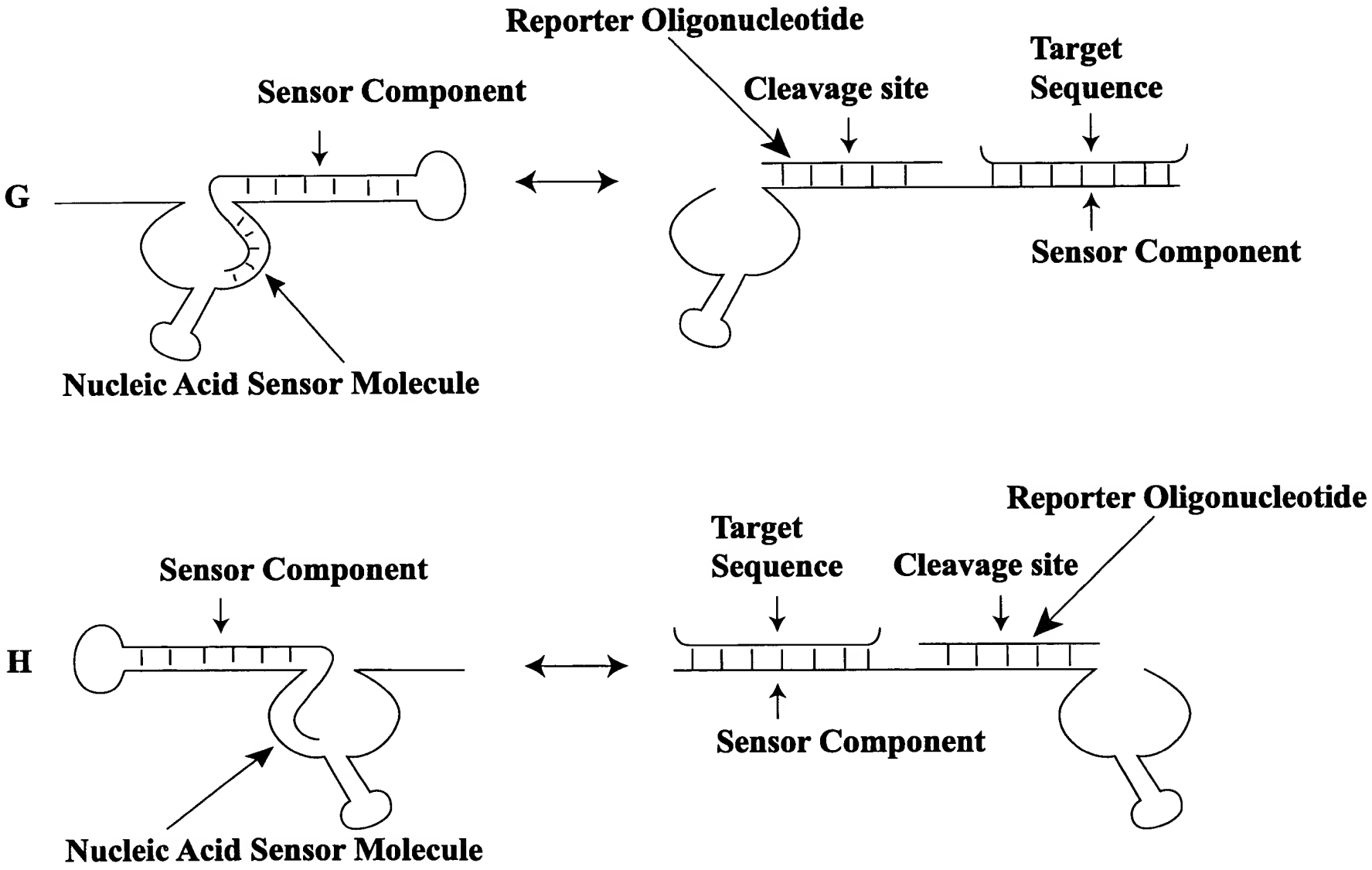


Figure 9. Examples of Diagnostic Effector Molecules

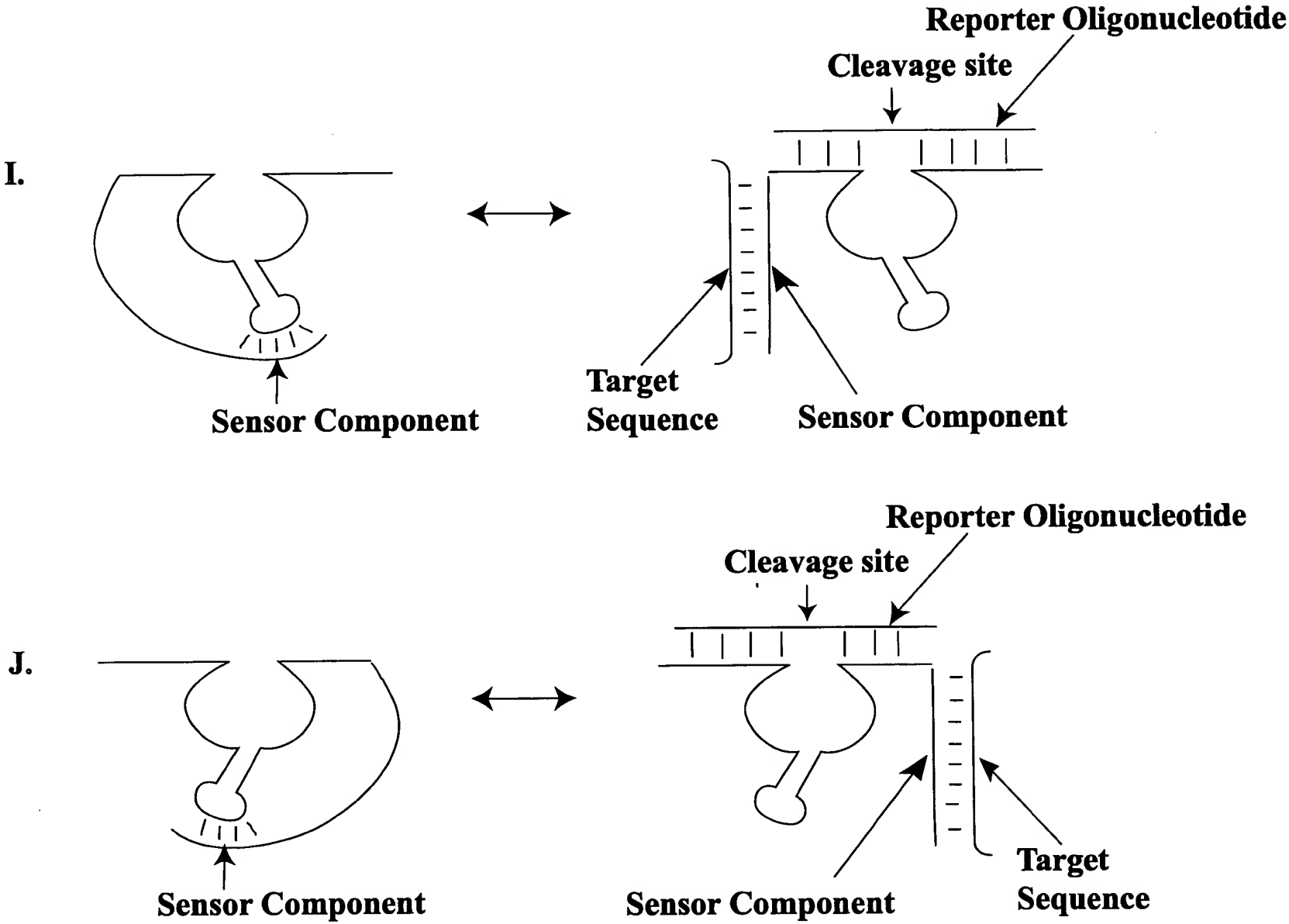
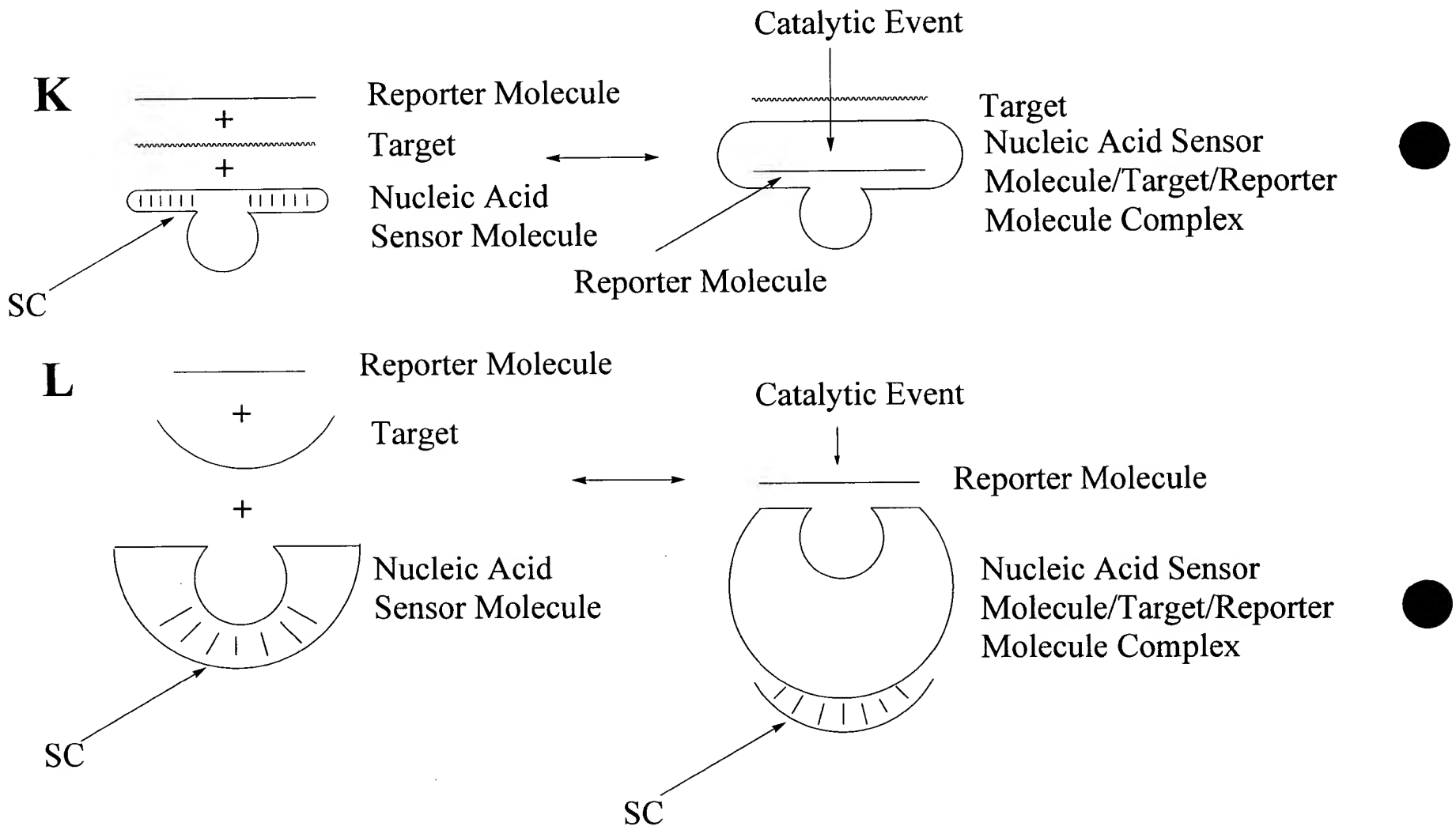


Figure 10: Examples of Diagnostic Effector Molecules



SC = Sensor Component

Figure 11: Examples of Diagnostic Effector Molecules

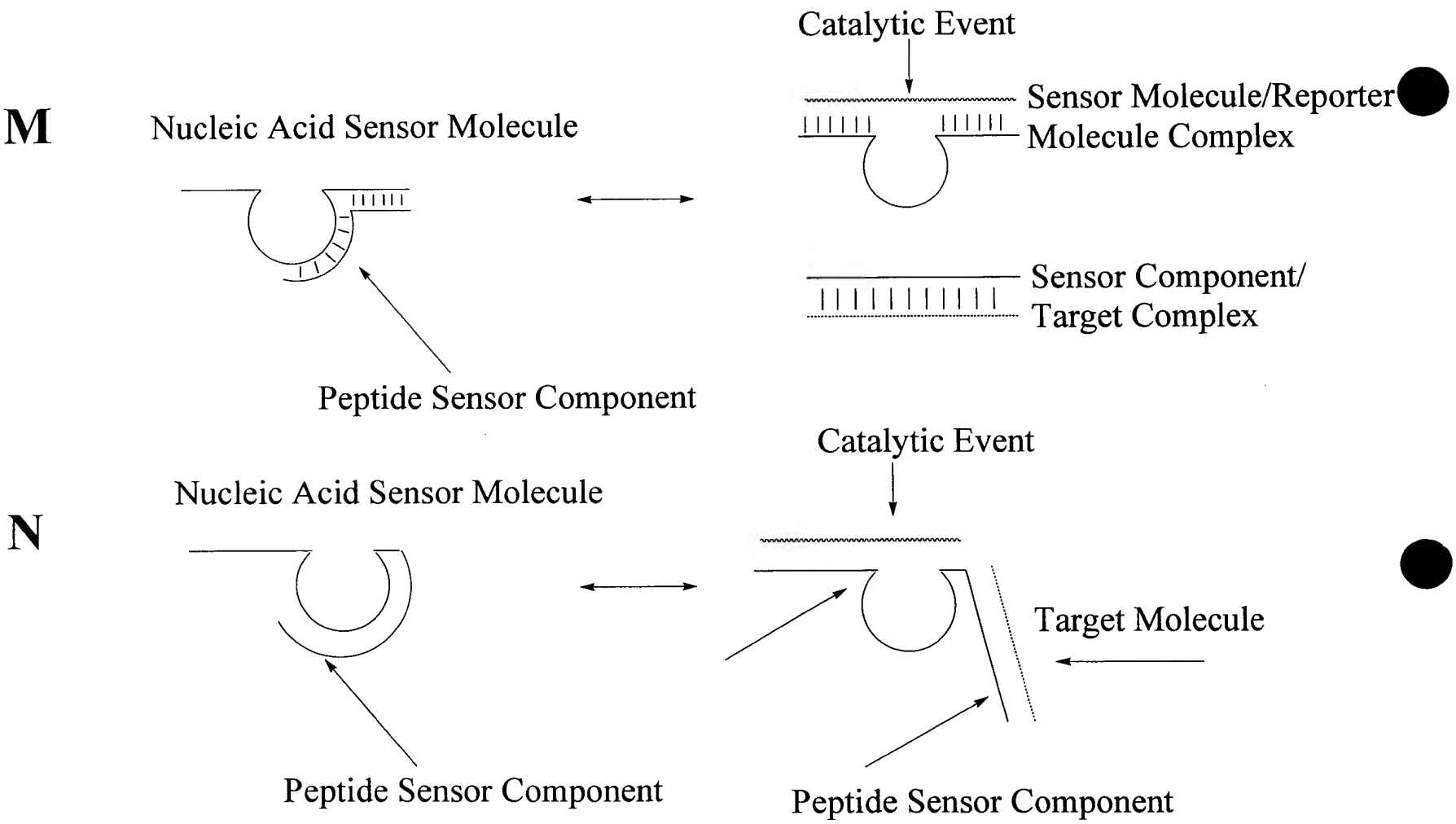
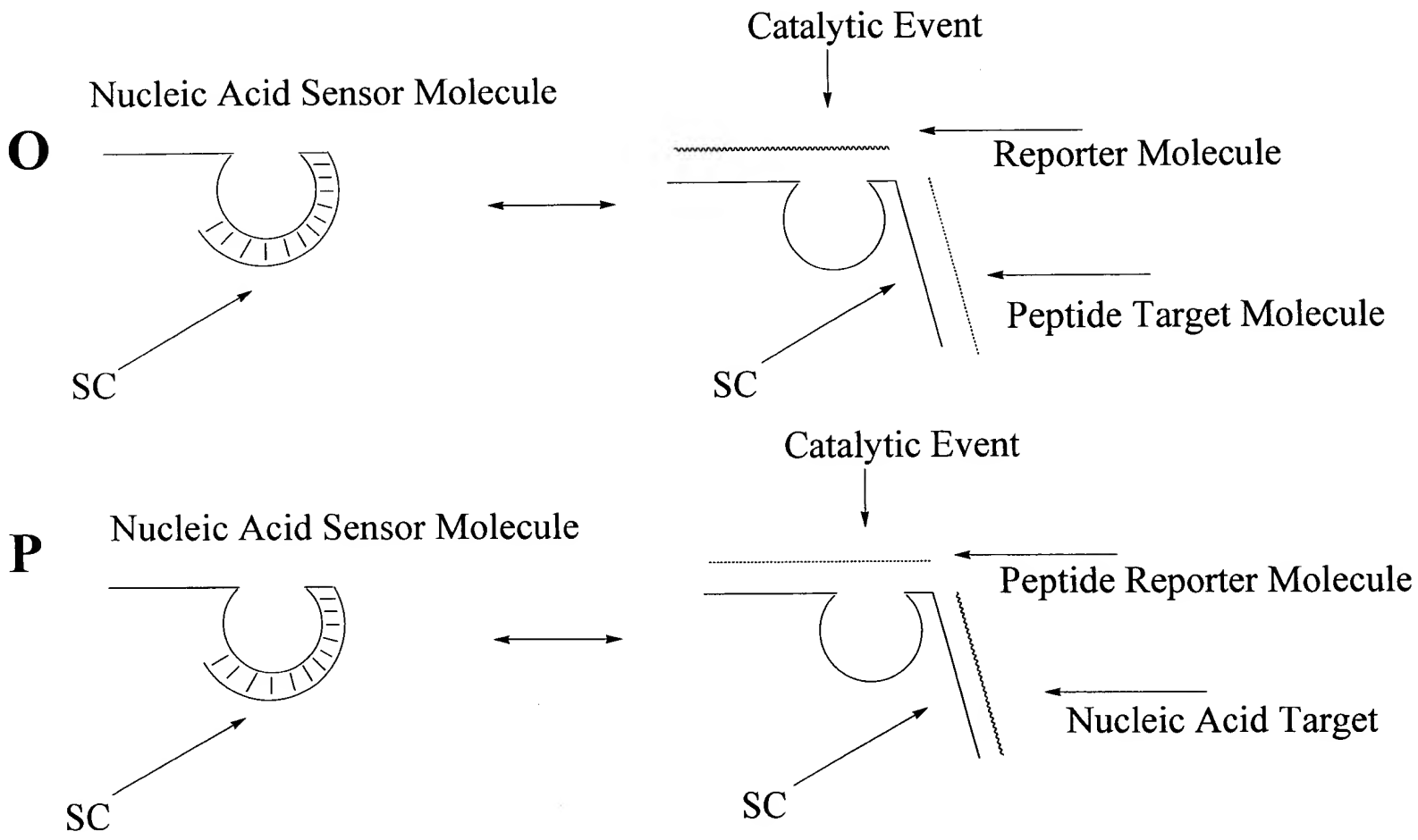


Figure 12: Examples of Diagnostic Effector Molecules



SC = Sensor Component

Figure 13: Examples of Diagnostic Effector Molecules

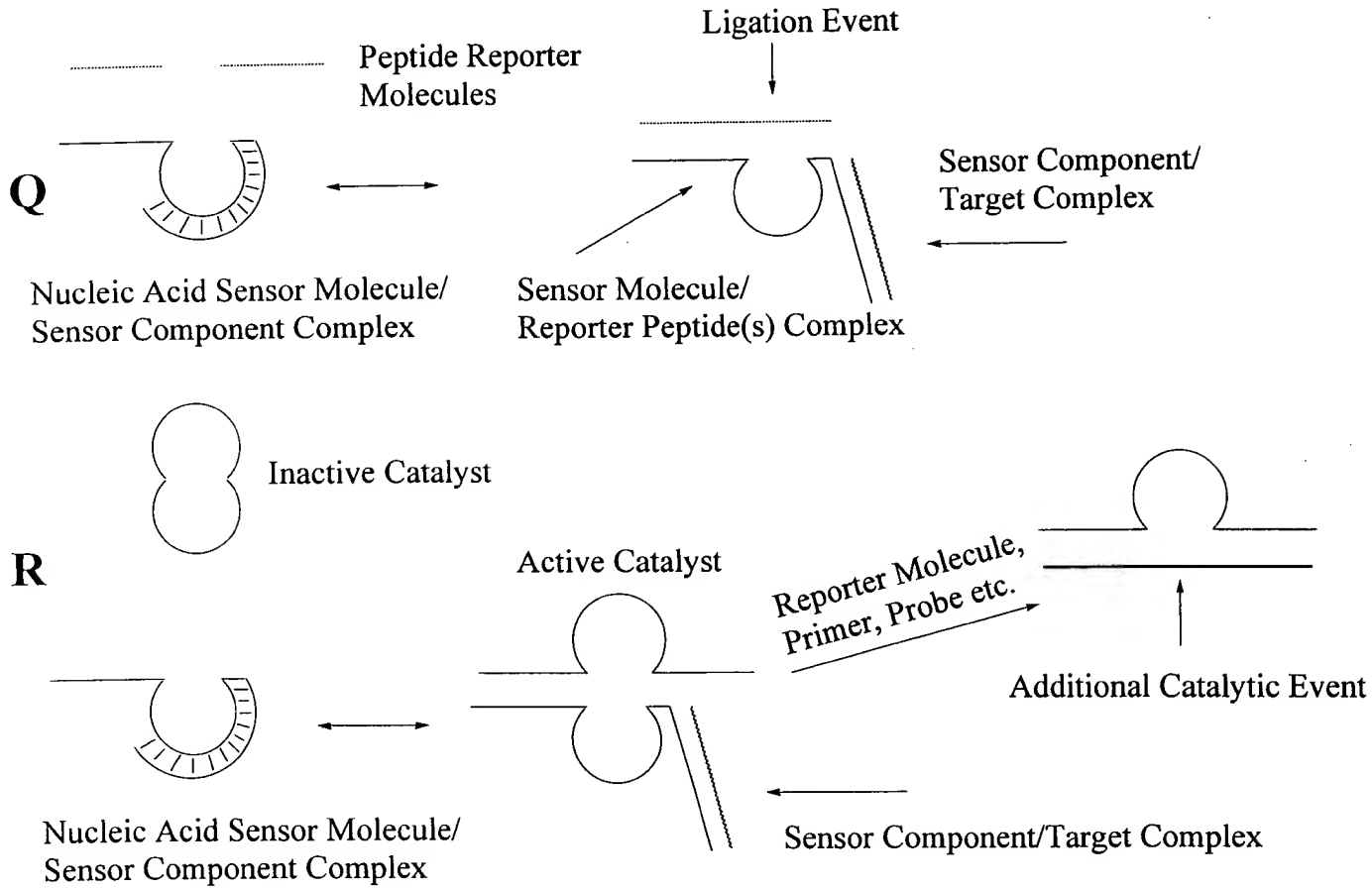


Figure 14: Inherent Amplification of Signal

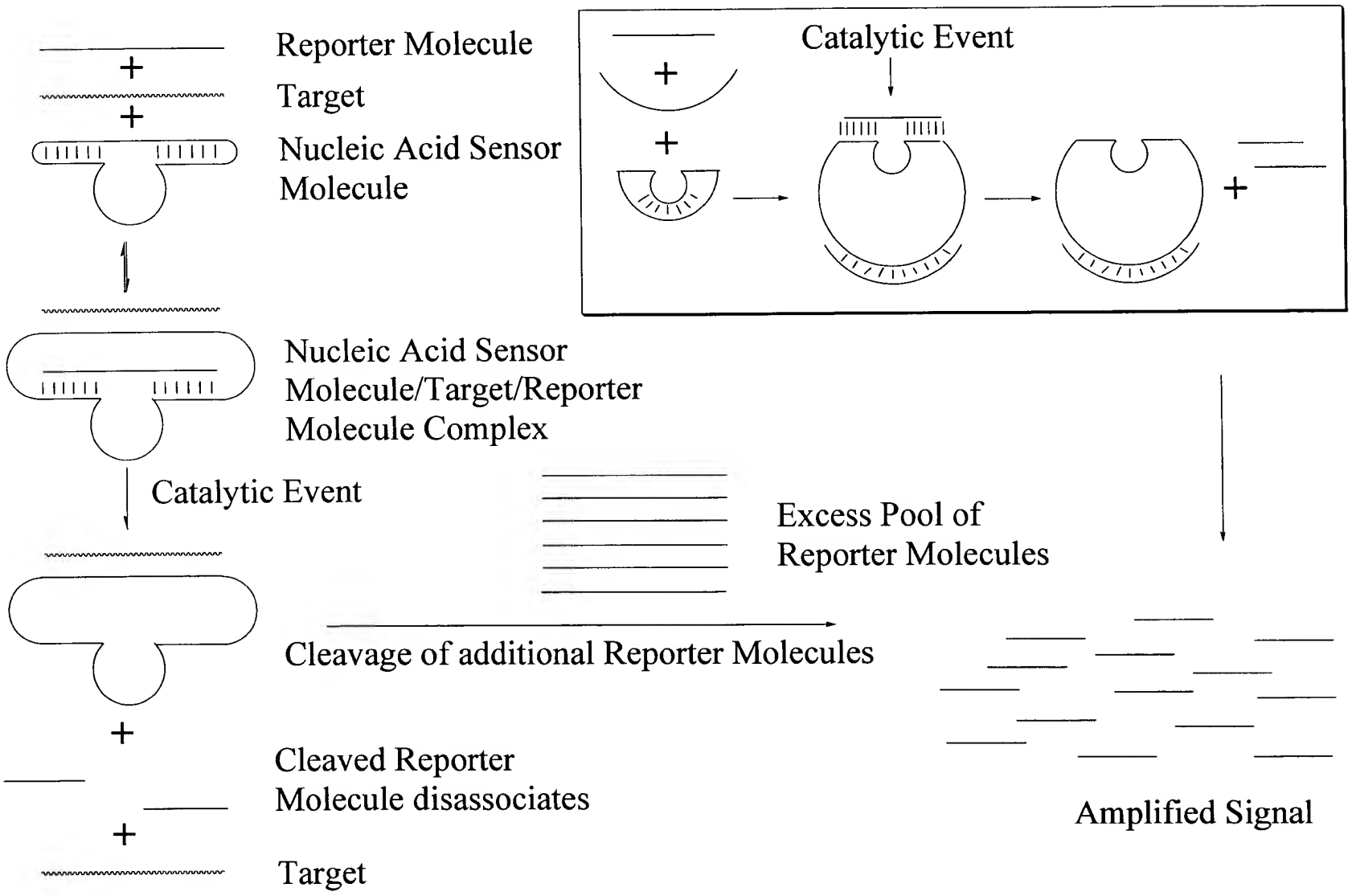


Figure 15: Example of Diagnostic System

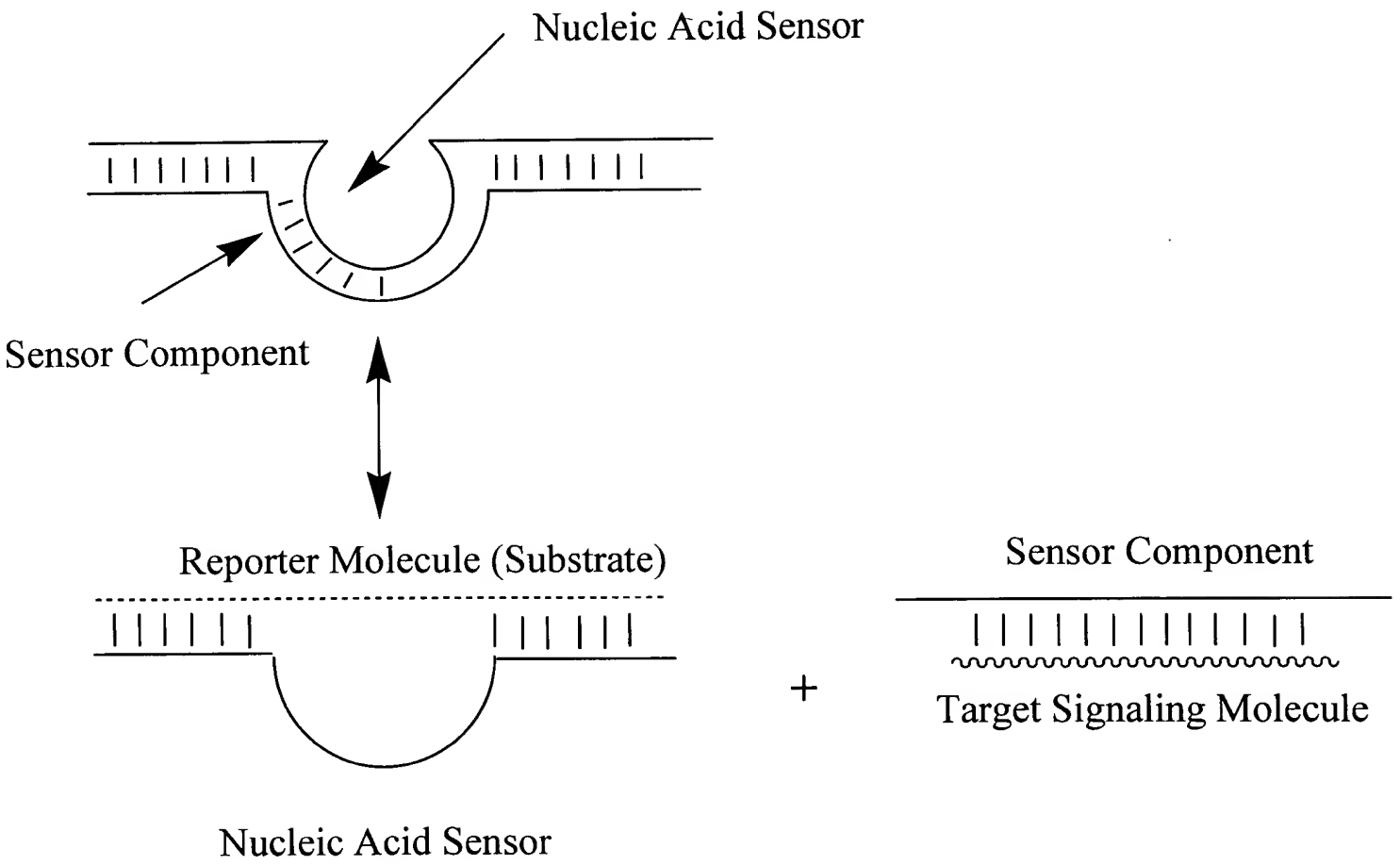


Figure 16: Ribozyme Diagnostic Screen

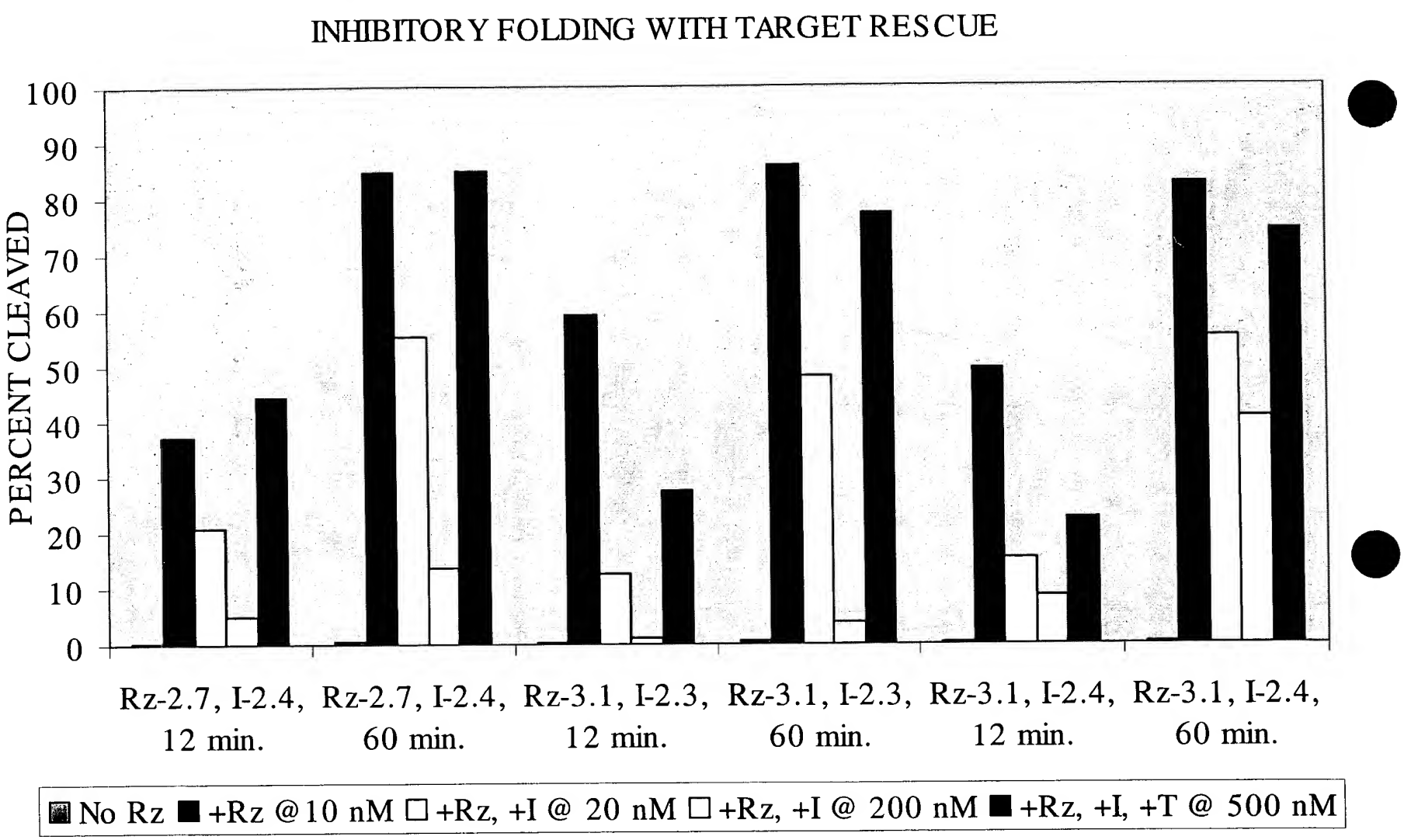


Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme

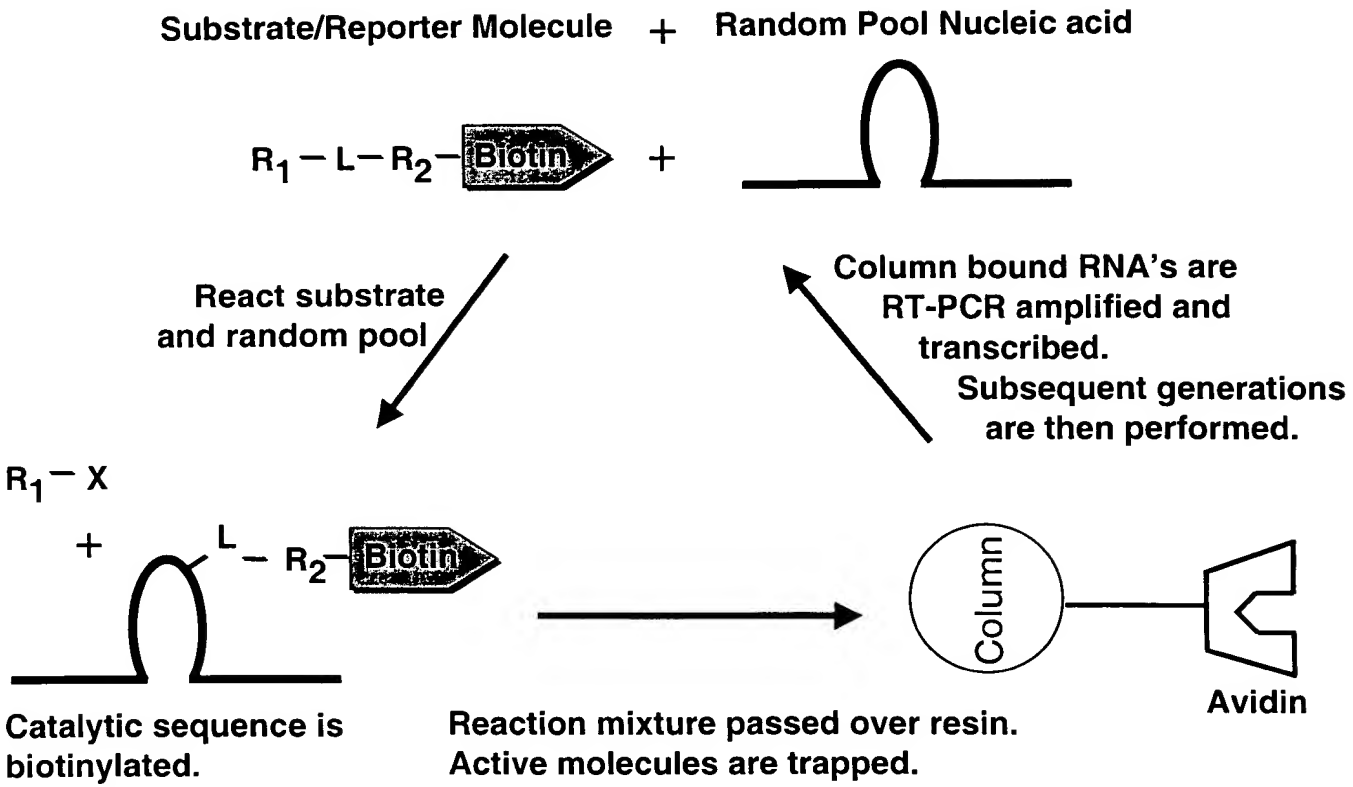


Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

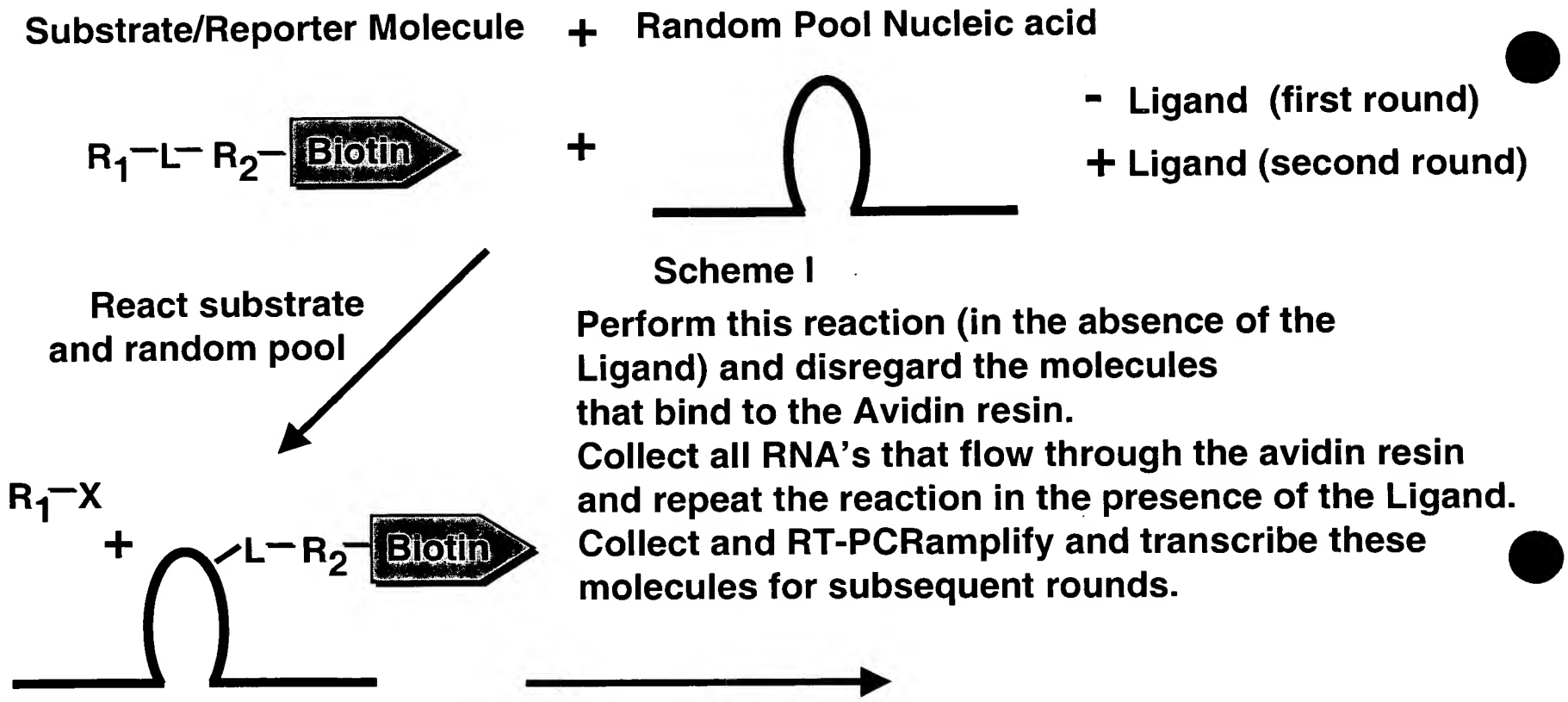


Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules- Ligand dependent

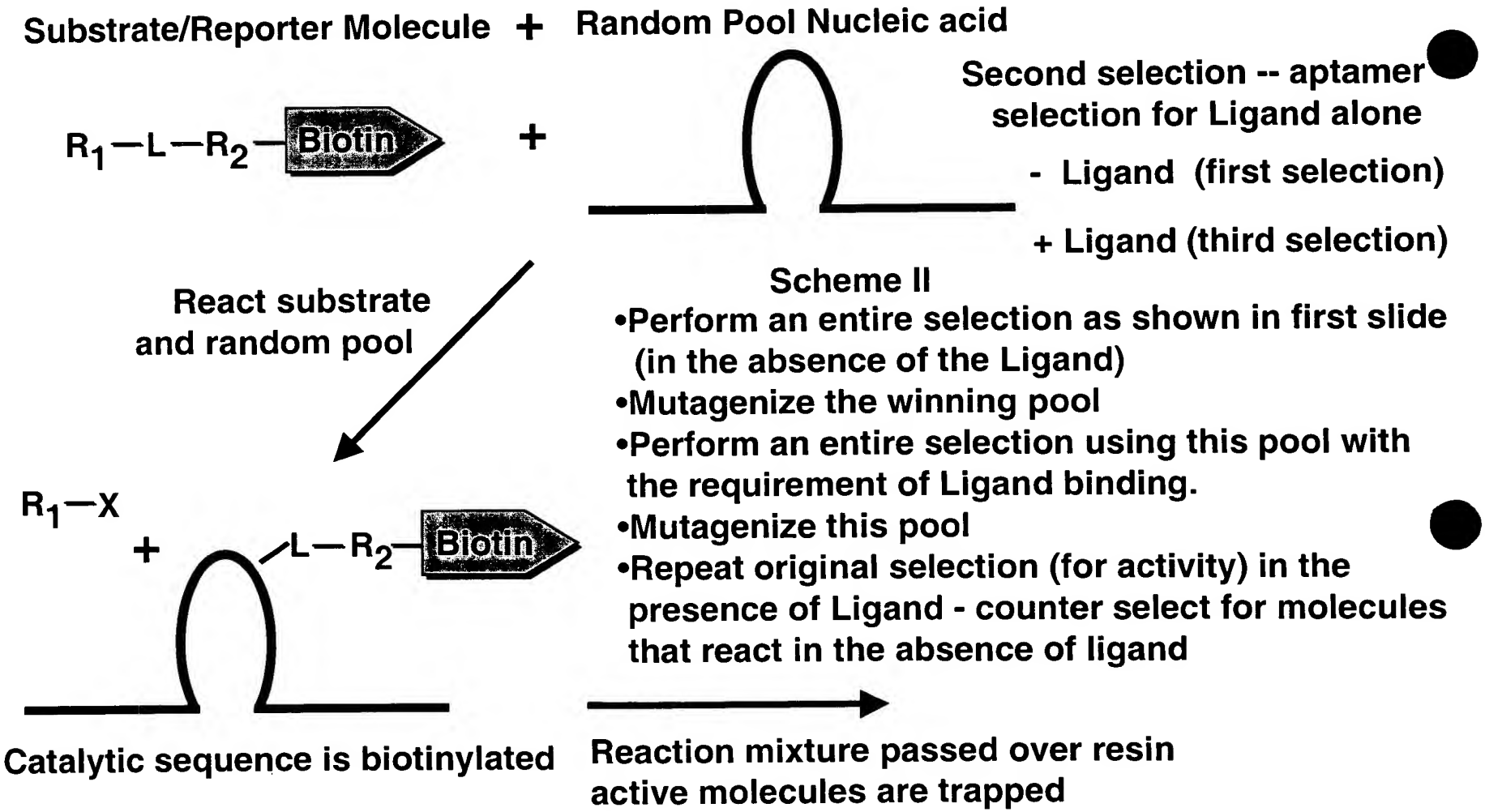
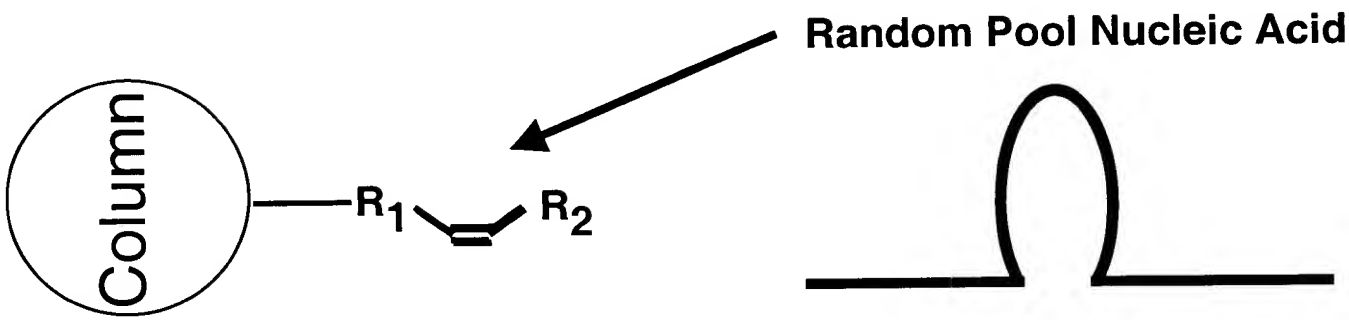
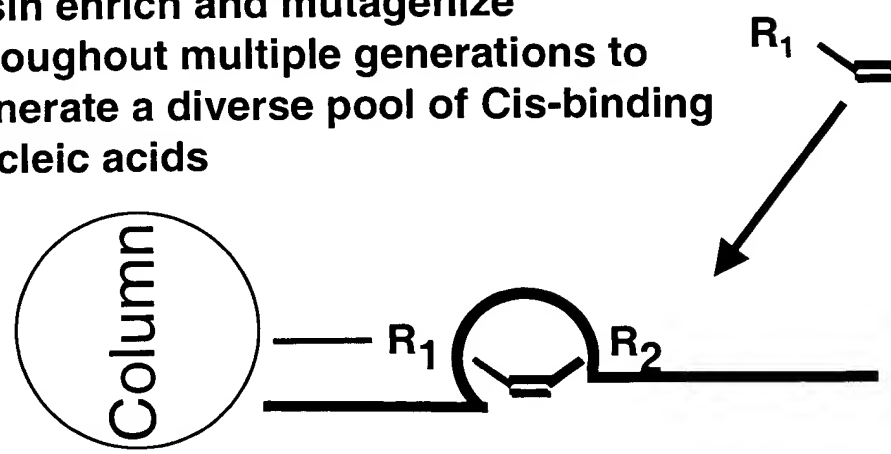


Figure 18a: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



Pass random pool over Cis-isomer-resin enrich and mutagenize throughout multiple generations to generate a diverse pool of Cis-binding nucleic acids



Cis-binding nucleic acids are then loaded on the resin and the Trans isomer is used to elute nucleic acids that bind the product tighter than the substrate in the reaction below

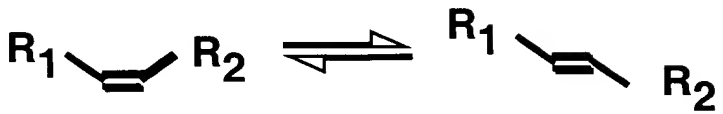
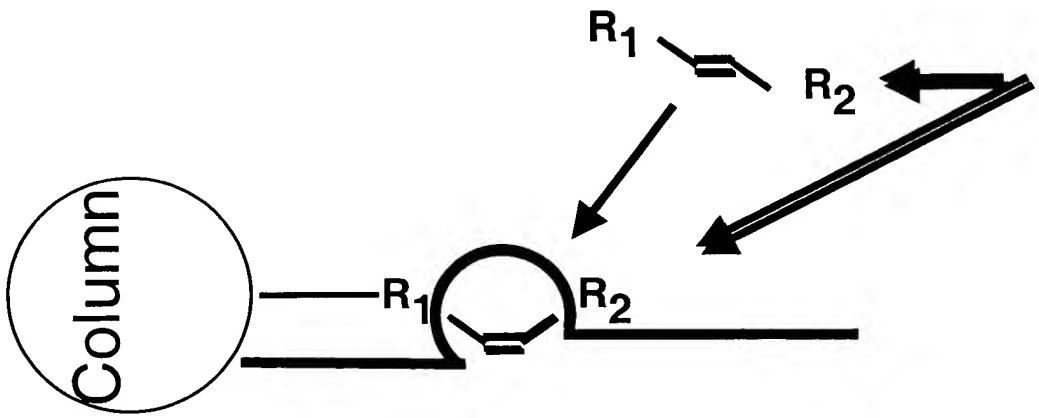


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



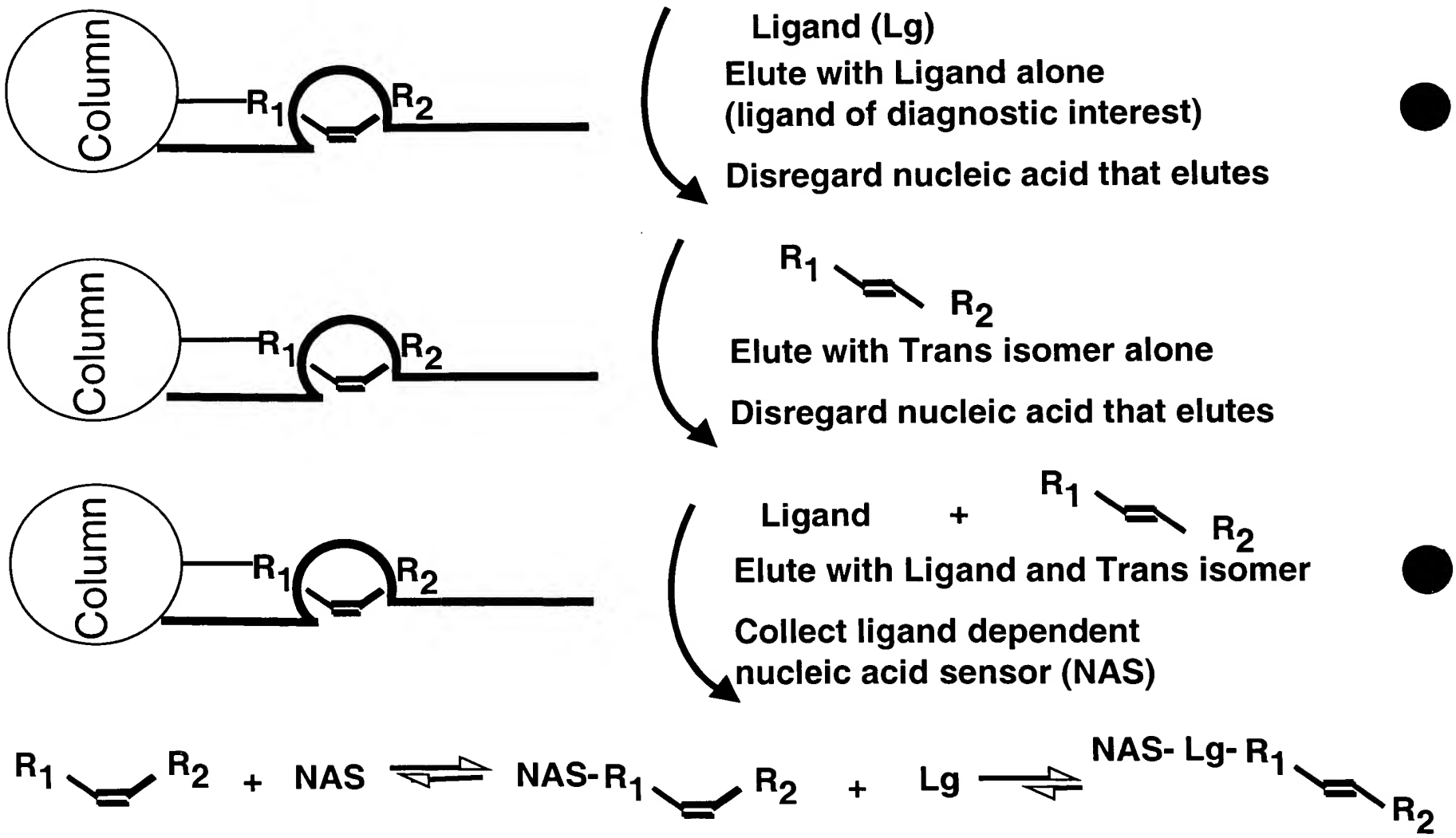
The concentration of ligand on the resin and the concentration of eluant ligand can be manipulated in order to bias for enzymatic nucleic acids that prefer one isomer over the other -and can therefore drive the reaction in a desired direction.

E.g. Selection for Cis-isomer at 100 uM - yeild $^{cis}K_d$ = 100 Um

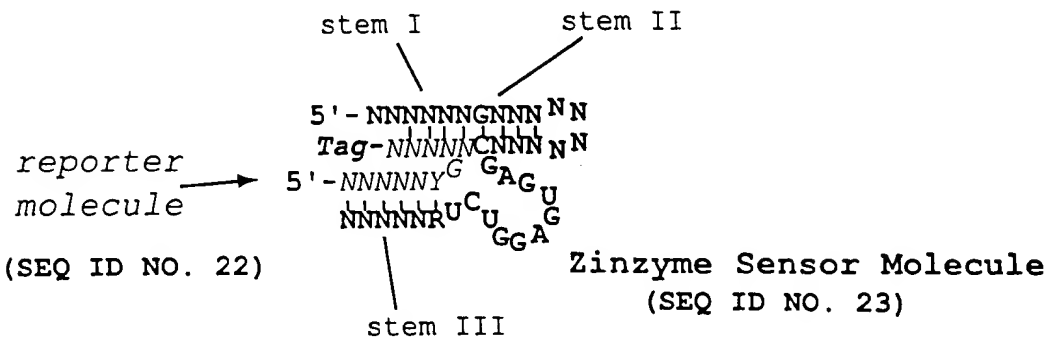
Elute with Trans-isomer at 0.1 uM - yield $^{trans}K_d$ = 0.1 uM

Isolate catalysts for the reaction below

Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent

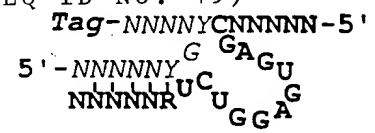


Zinzyme Sensor Molecule for detection of Nucleic Acid



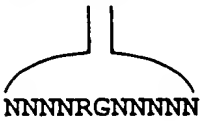
Inactive Zinzyme sensor/ reporter molecule complex

(SEQ ID NO. 49)



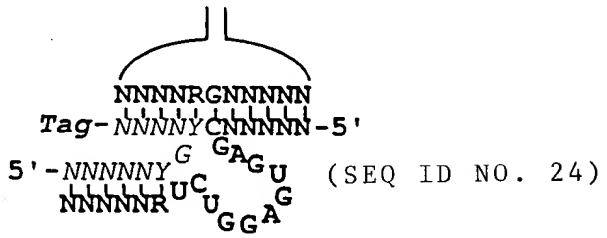
(SEQ ID NO. 24)

Target Signaling Molecule



Target Signaling Molecule

(SEQ ID NO. 25)

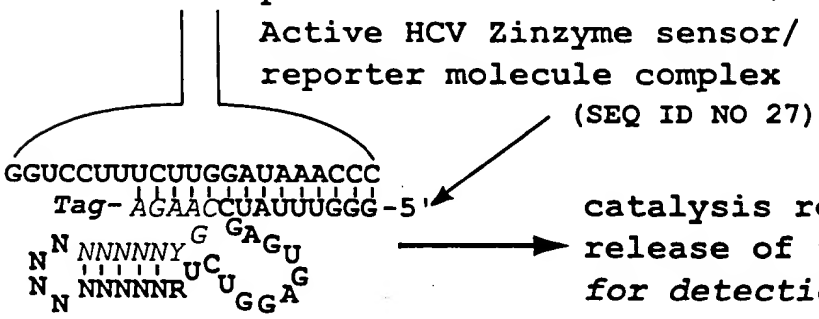


Active Zinzyme sensor/ reporter molecule complex

Stem-loop III of HCV (SEQ ID NO. 26)

Active HCV Zinzyme sensor/ reporter molecule complex

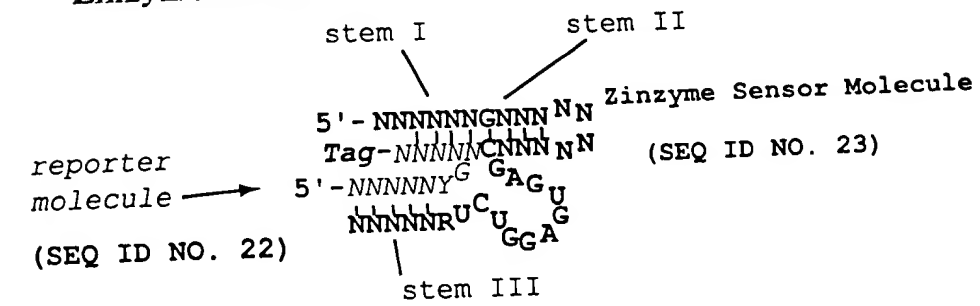
(SEQ ID NO 27)

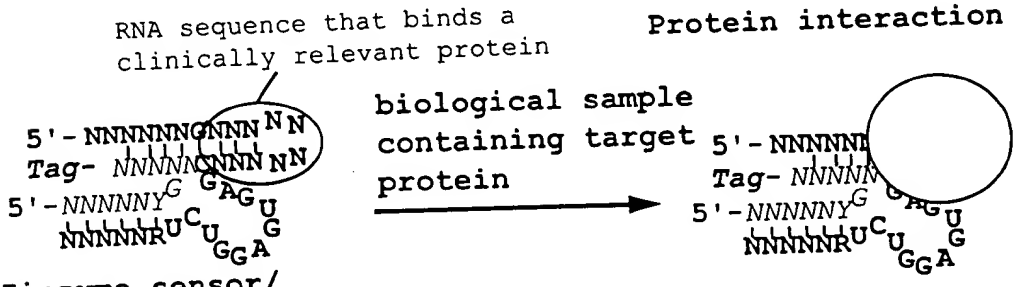


Zinzyme sensor can be attached to solid support/surface, for example at the 5'-end

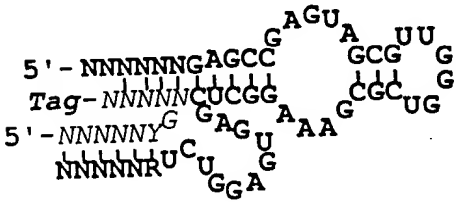
FIG. 19

Zinzyme Sensor Molecule for detection of Protein

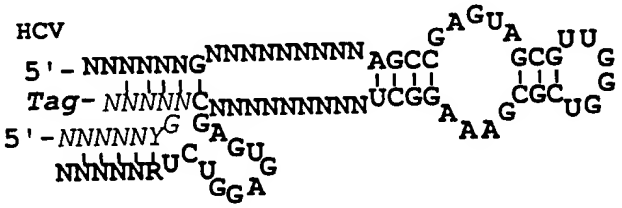




Sensor/reporter complex for detection of HCV core protein



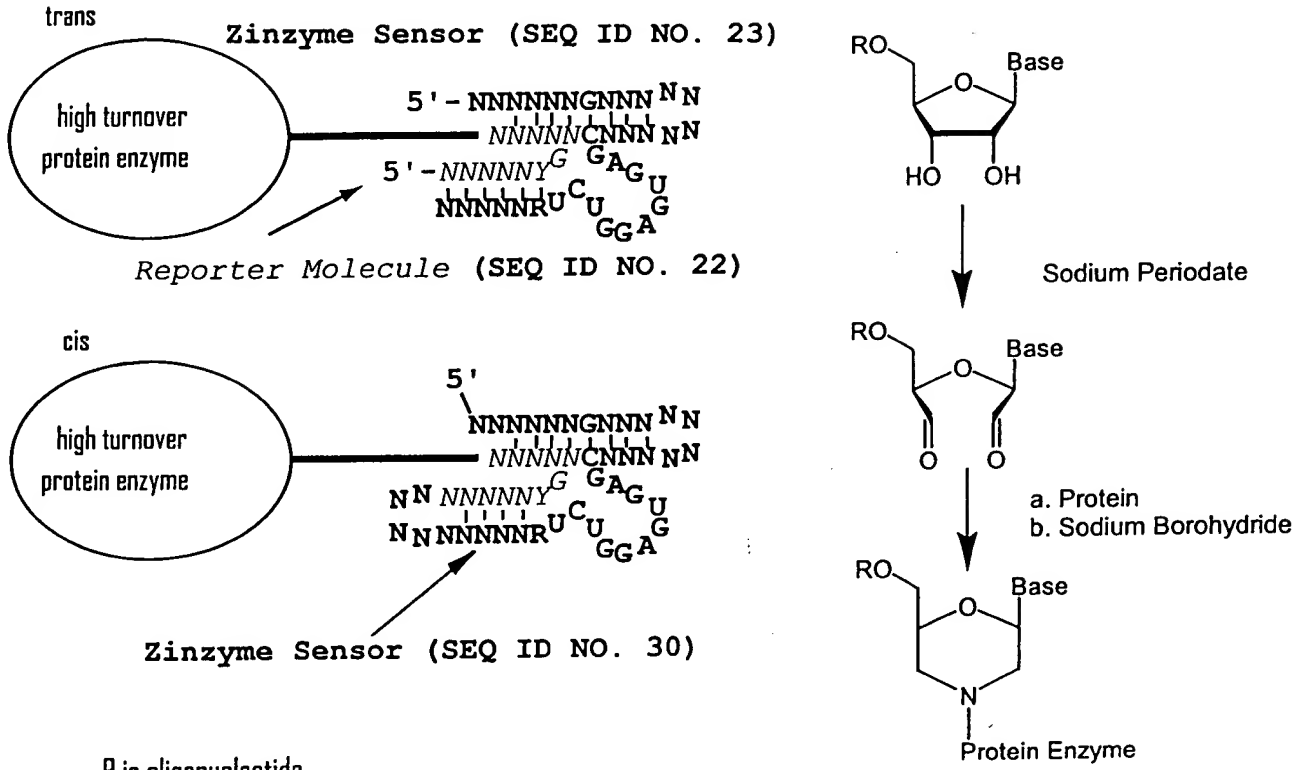
HCV Zinzyme sensor with loop IIID of HCV (directs the binding of HCV core protein)
(SEQ ID NO 28)



HCV Zinzyme sensor with loop IIID of HCV connected via randomized linker
(SEQ ID NO 29)

FIG. 20

Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.
Protein can be attached via amino linker.
Alternately, R is phorphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

FIG. 21

Amplification of signal via use of protein enzyme conjugate

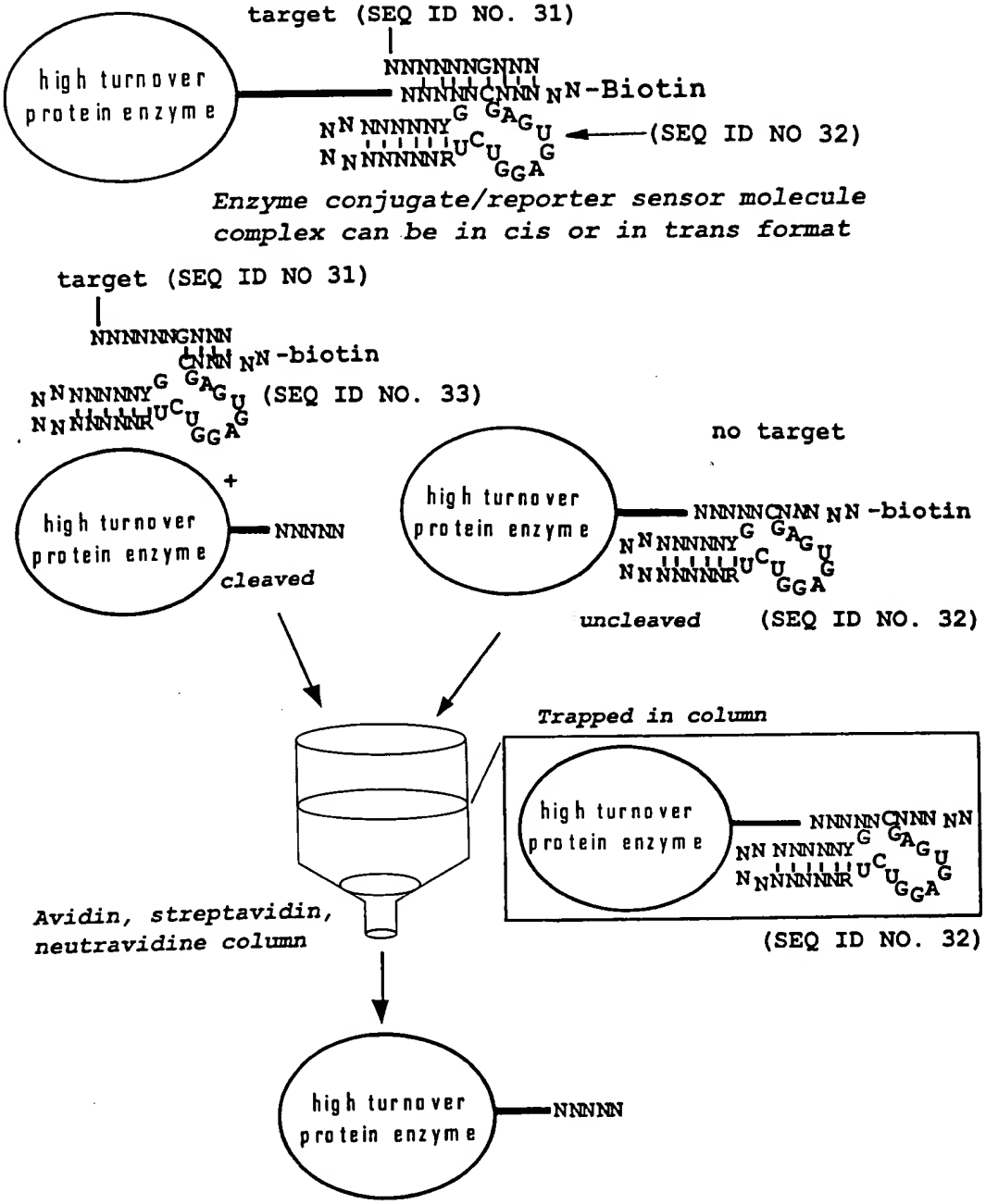
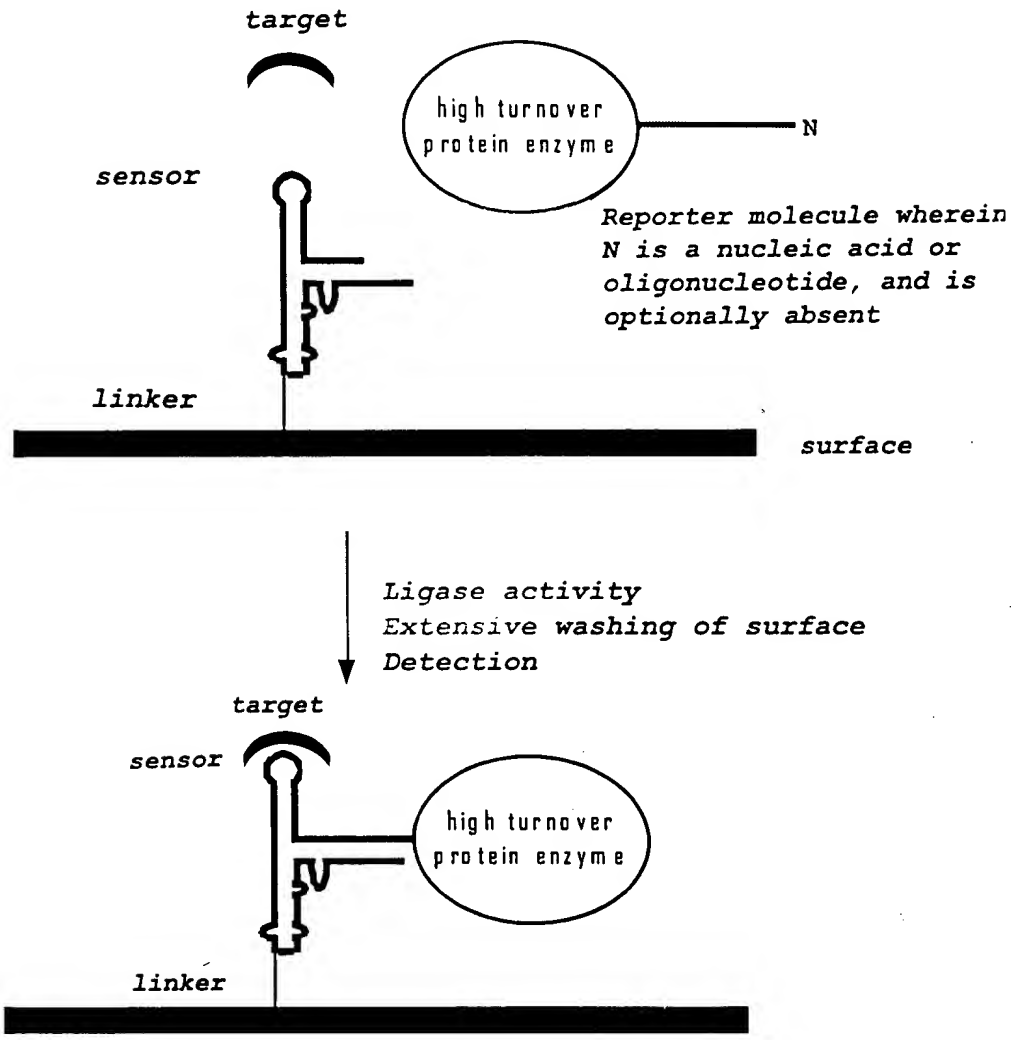


FIG. 22

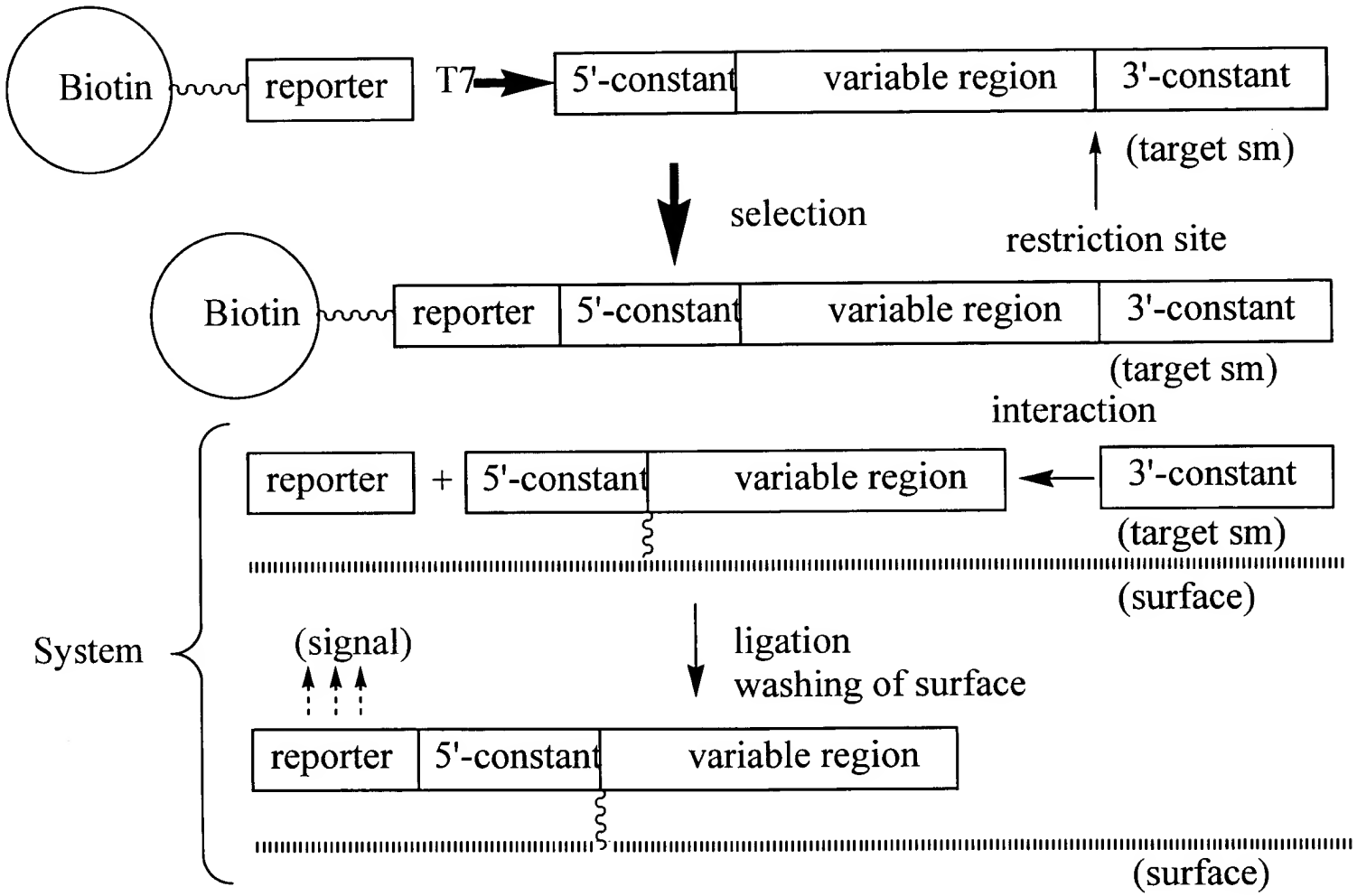
Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

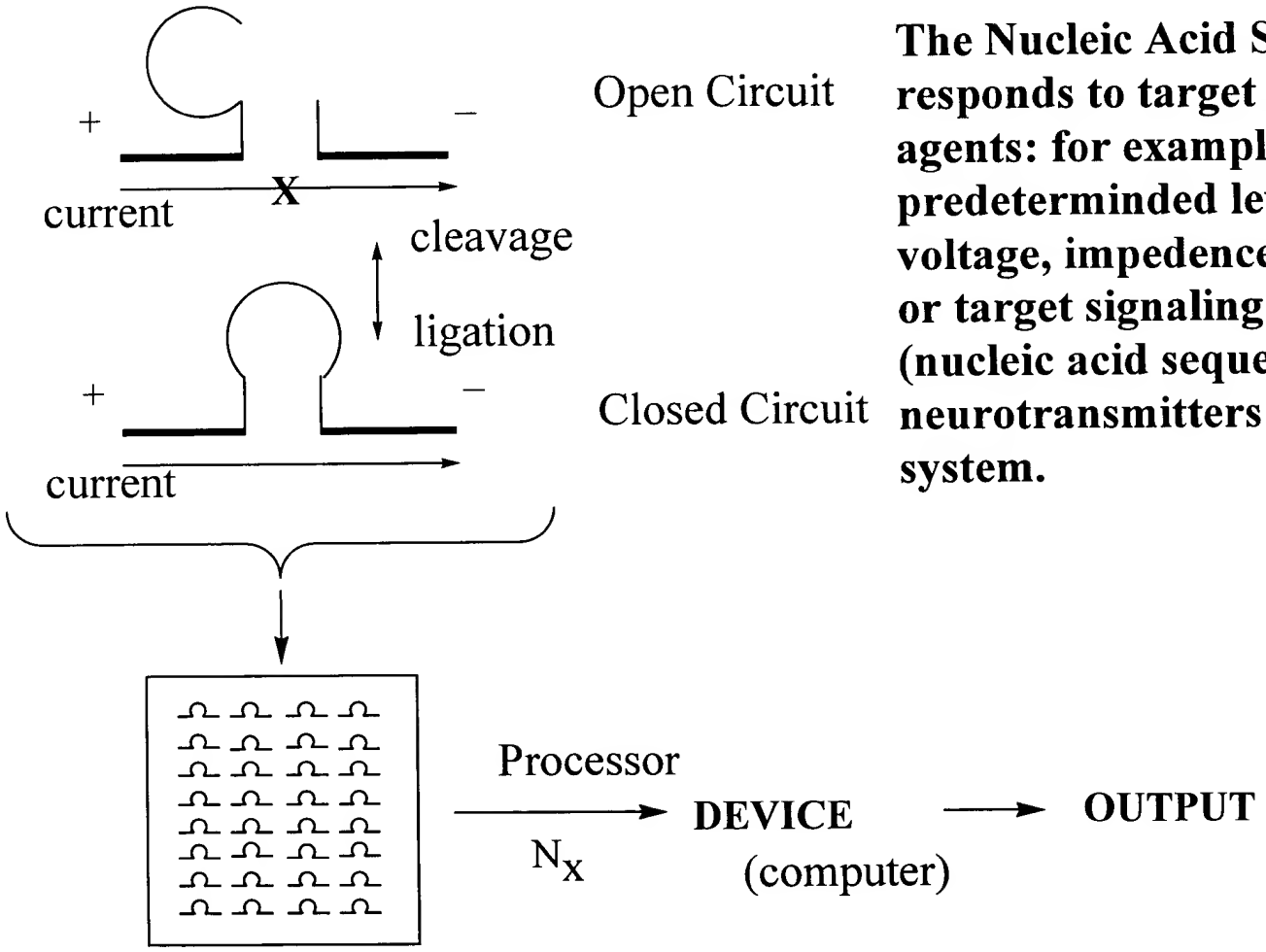
FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity



(sm= signaling molecule)

Figure 25: Nucleic Acid Sensor Molecule-Based Electric Cirucuit



The Nucleic Acid Sensor Circuit responds to target signaling agents: for example predeterminded levels of current, voltage, impedence, or target signaling molecules (nucleic acid sequences, peptides, neurotransmitters etc.) in a system.

FIG. 26 *Target Inactivation of Zinzyme Sensor Molecule*

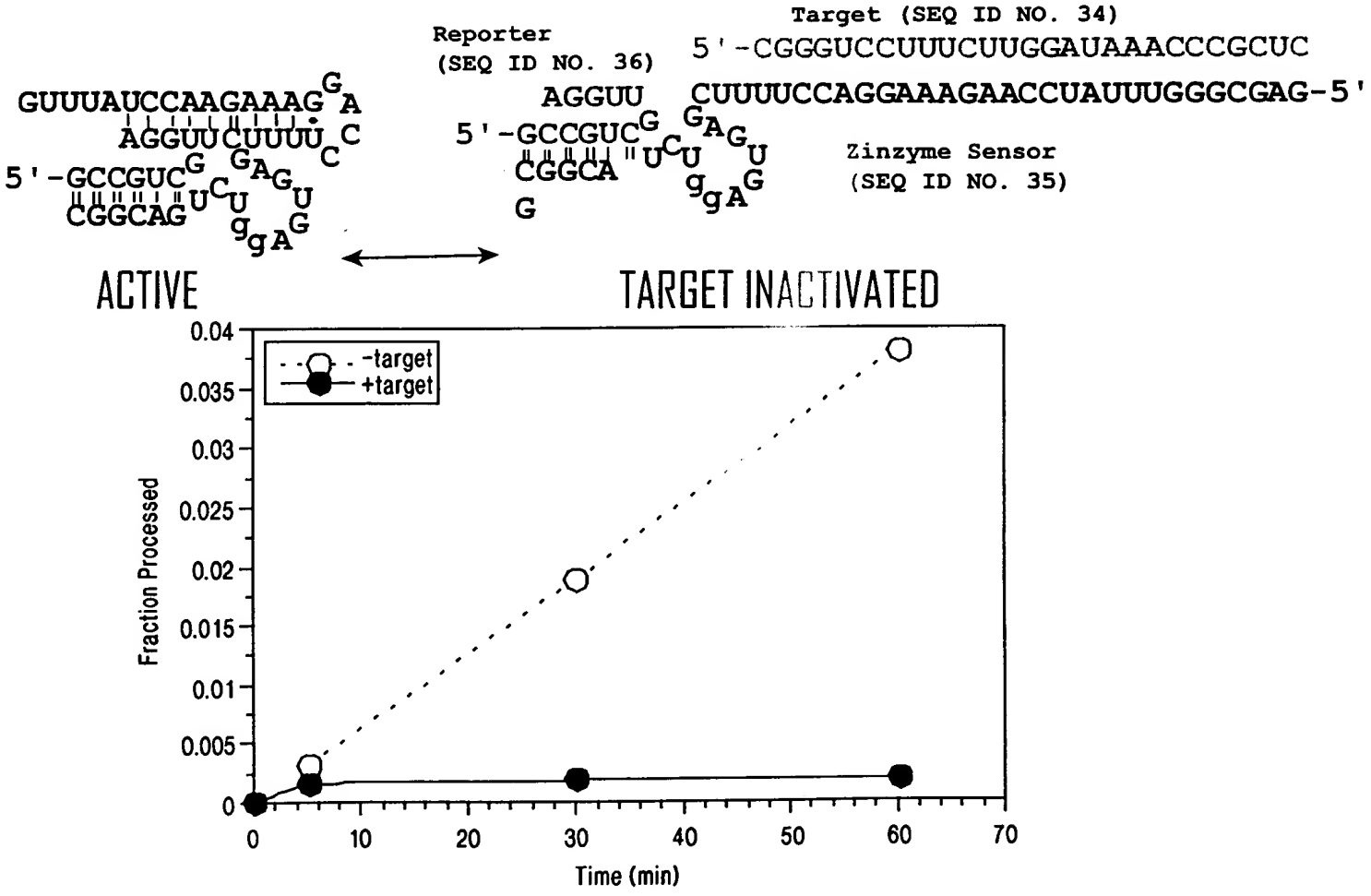
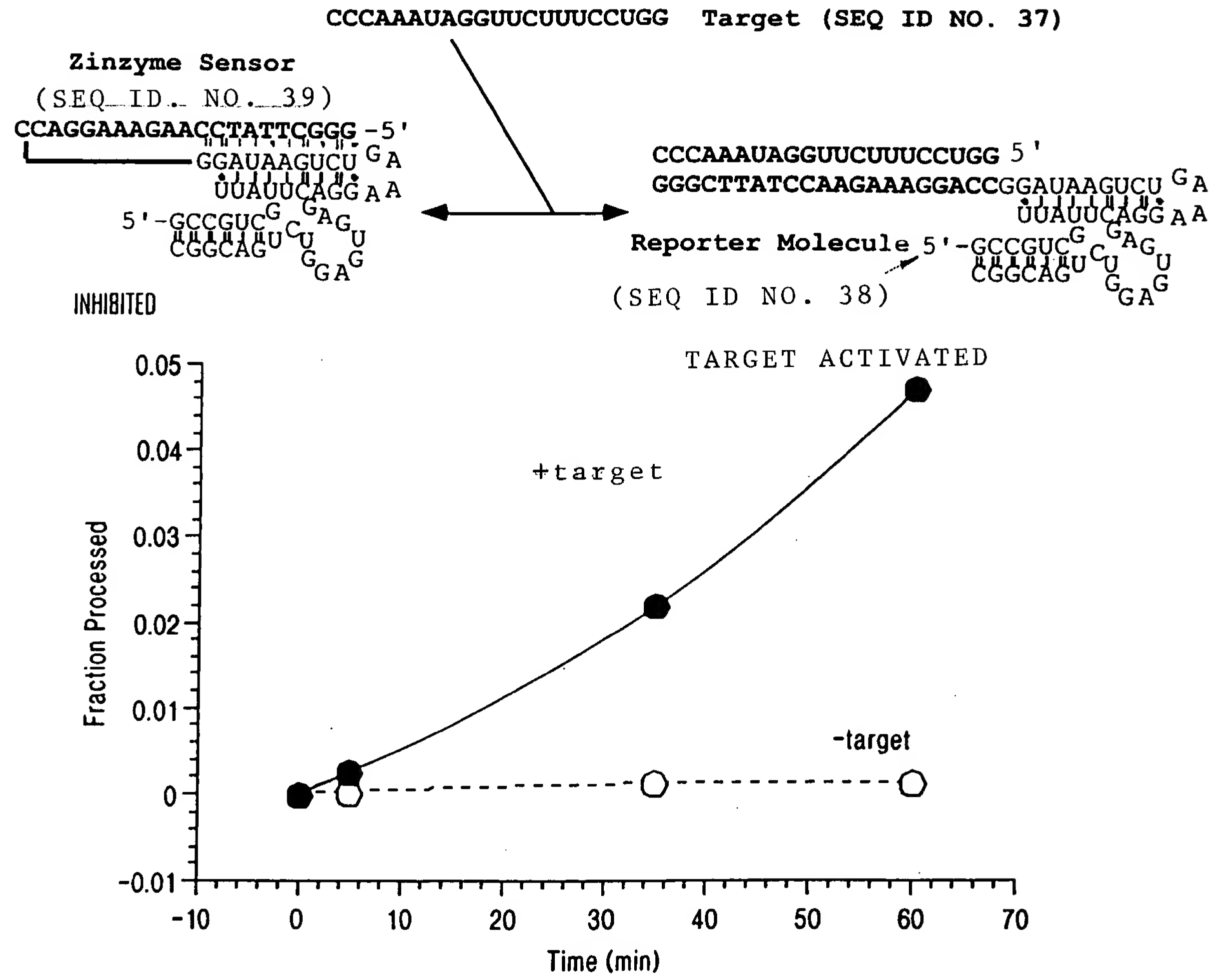
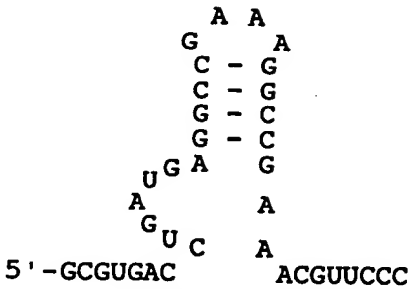


FIG. 27 *Target Activation of Zinzyme Sensor Molecule*

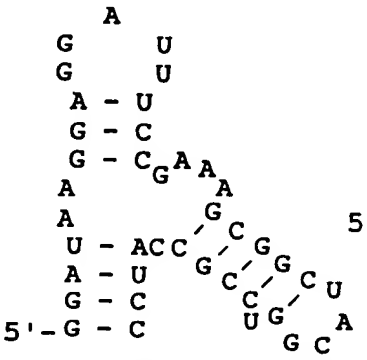


Erk modulated Nucleic Acid Sensor Molecule



HAMMERHEAD (HH)

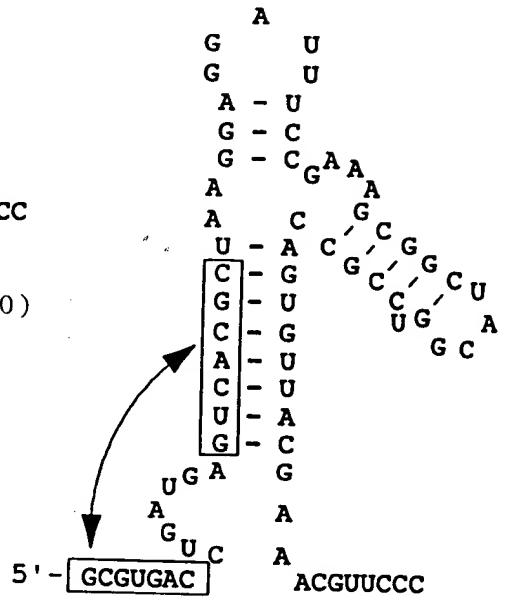
(SEQ ID. NO. 40)



ERK SENSOR COMPONENT

K_D =300 pM

(SEQ ID.NO. 41)



EHHI.3 sensor molecule

K_D =570 nM

(SEQ ID.NO. 42)

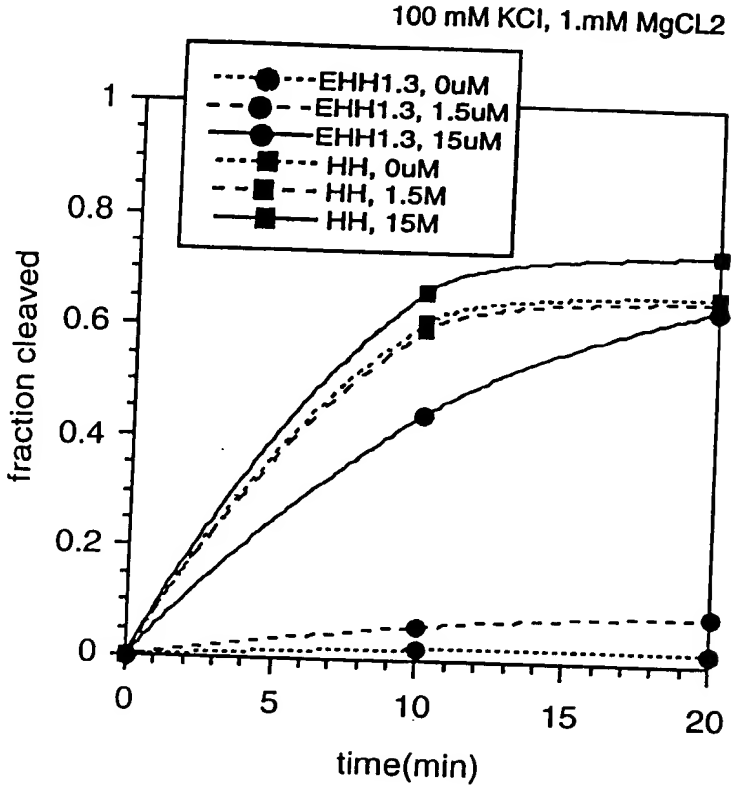


FIG. 28

Figure 29: Half-Zinzyme Construct

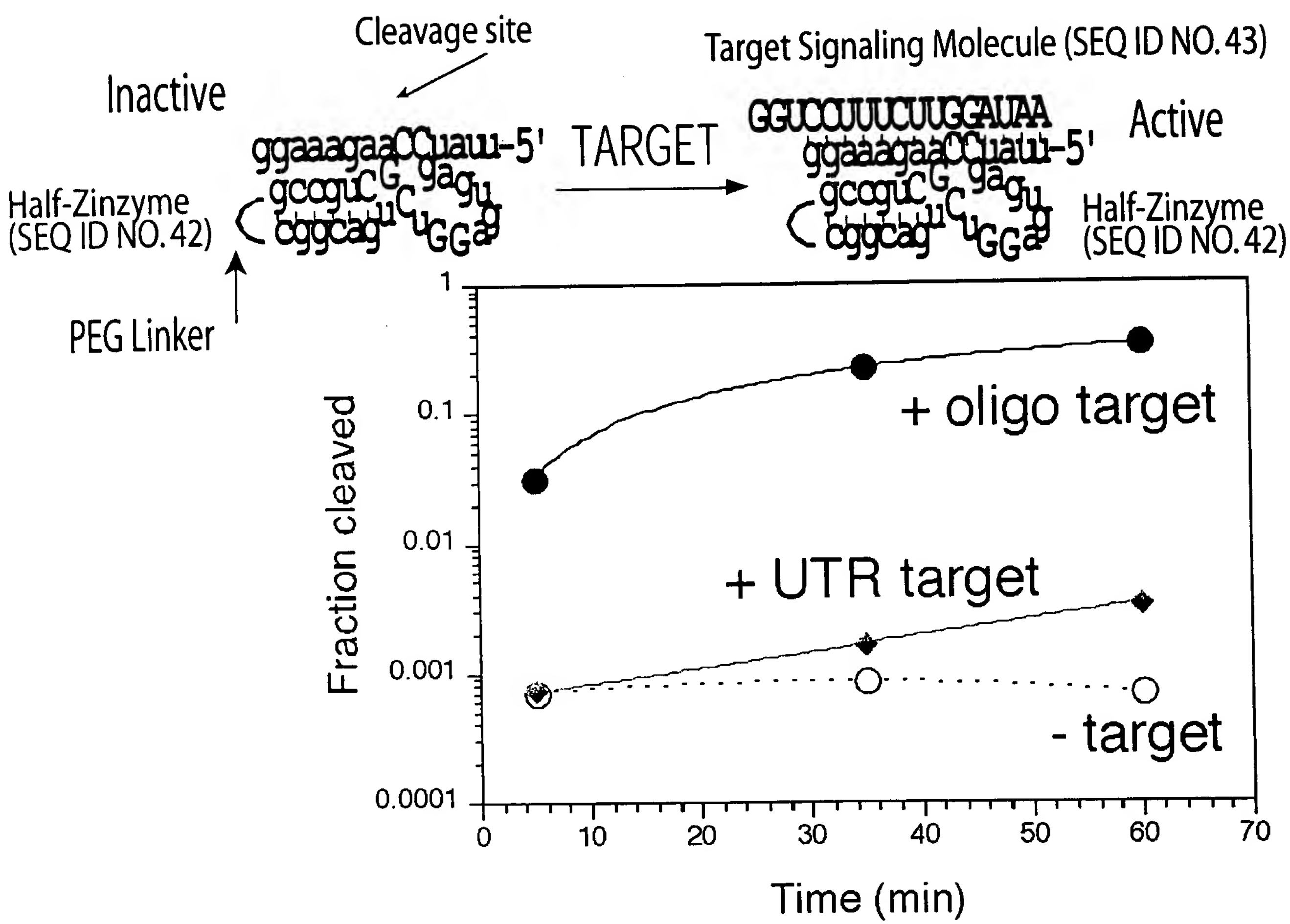
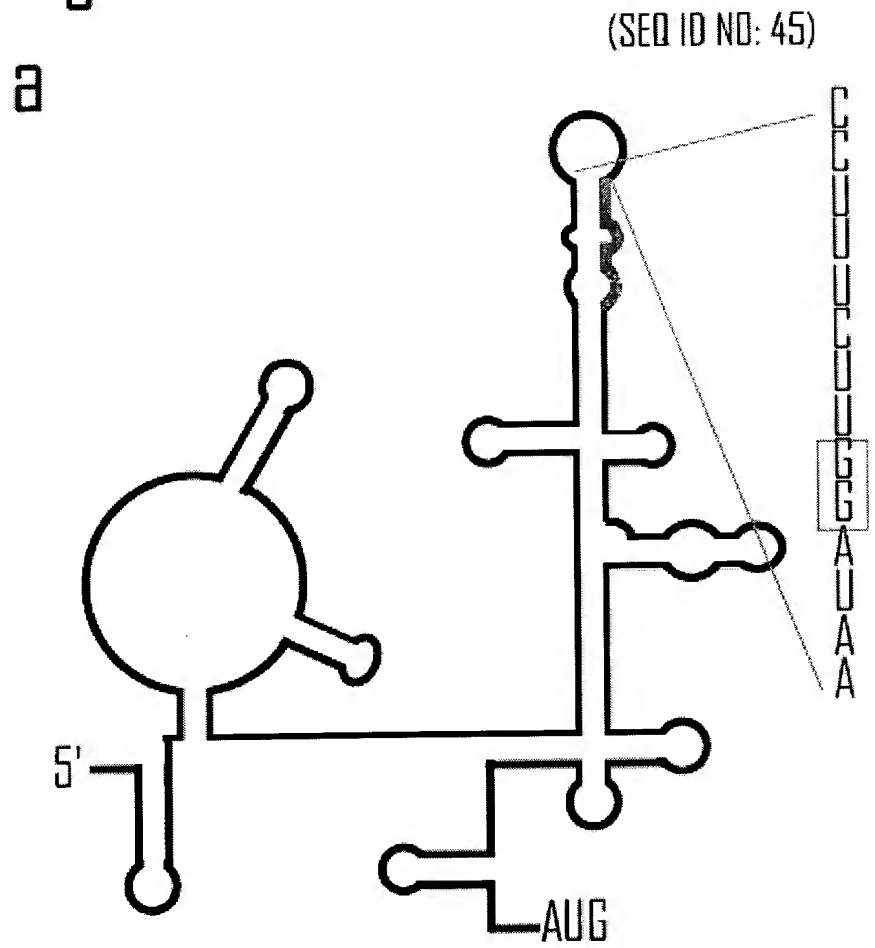


Figure 30


a
(SEQ ID NO: 45)
5'
AUG
CCUCUCCUGGAUAA

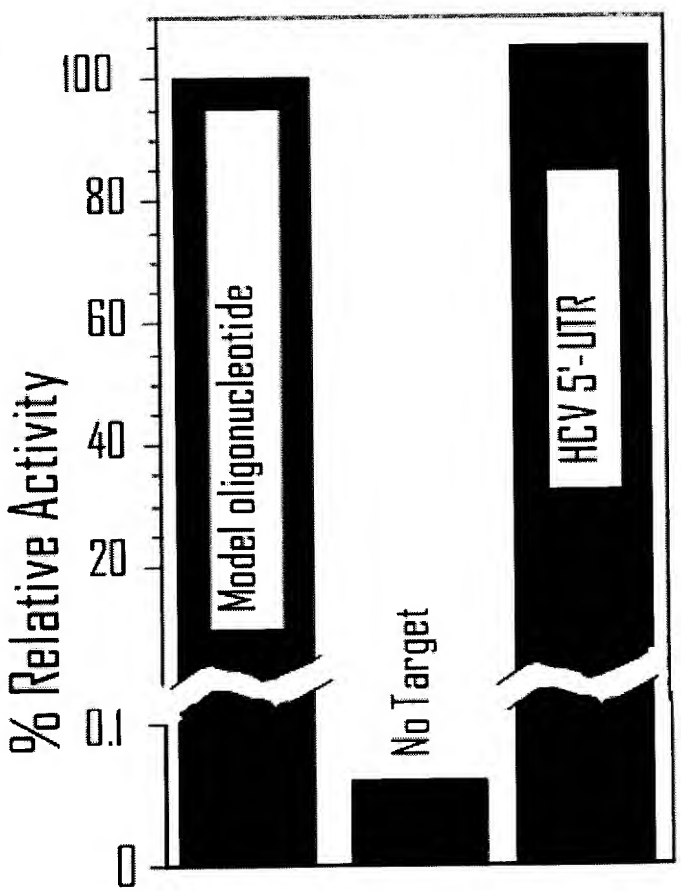

b
% Relative Activity
100
80
60
40
20
0.1
0
Model oligonucleotide
No Target
HCV 5'-UTR

Figure 31

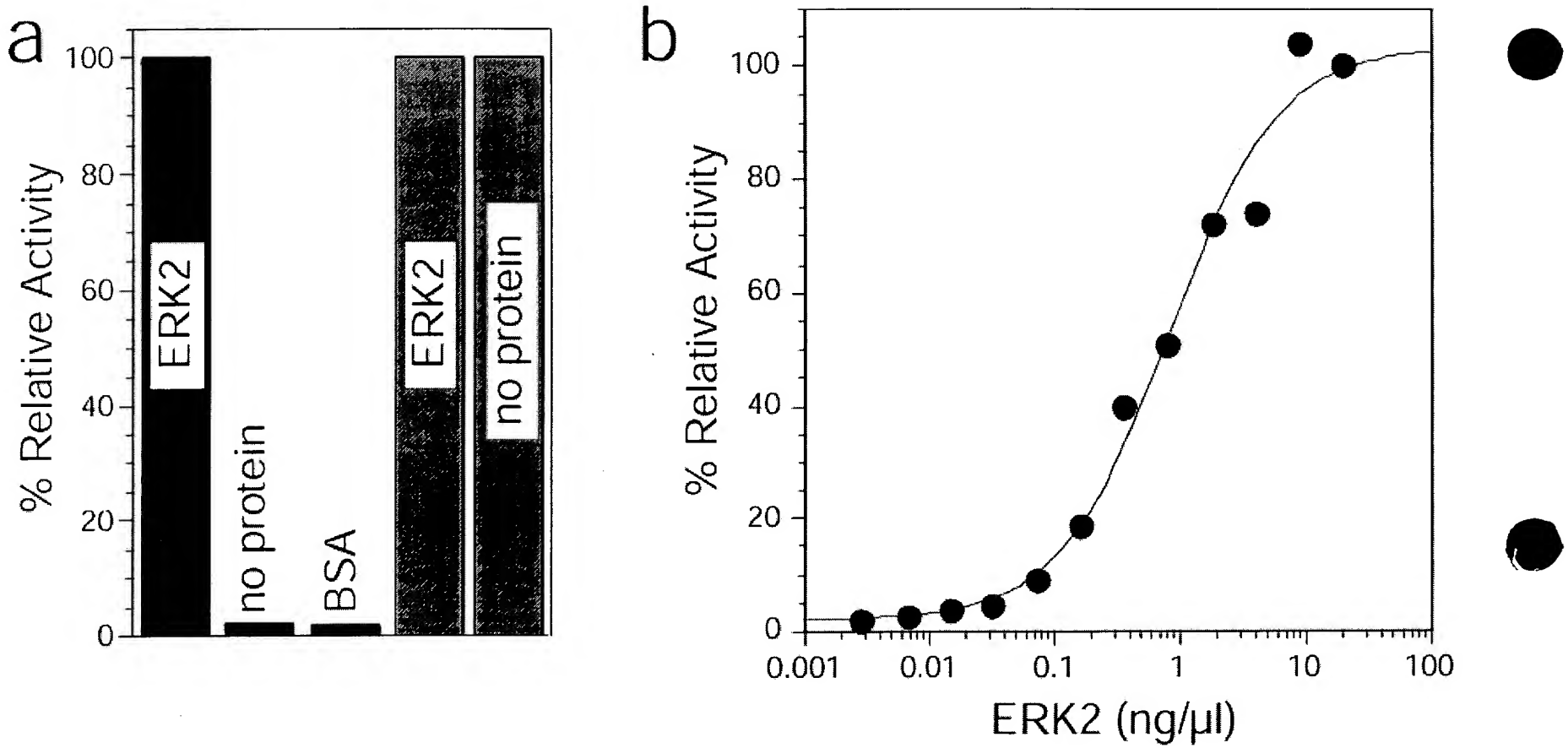


Figure 32

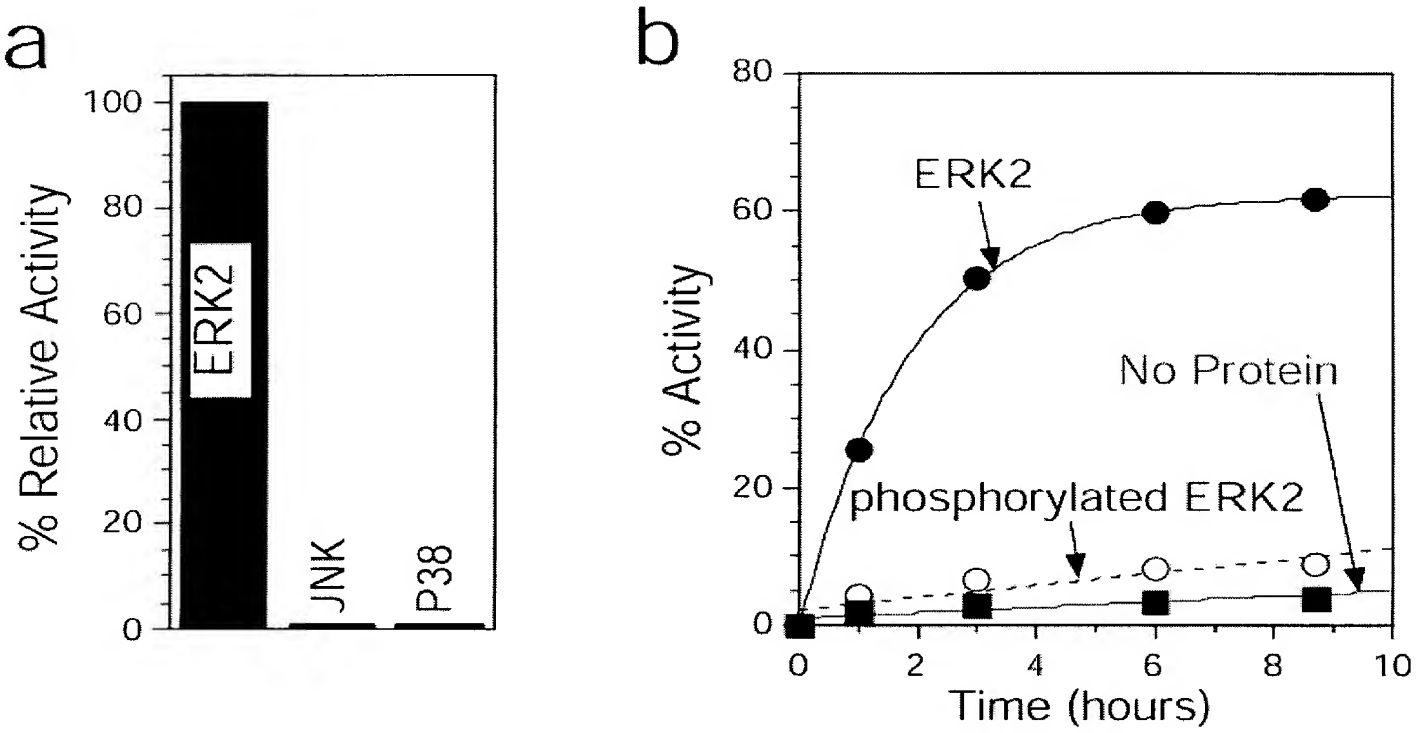


Figure 33: Halfzyme Ligase

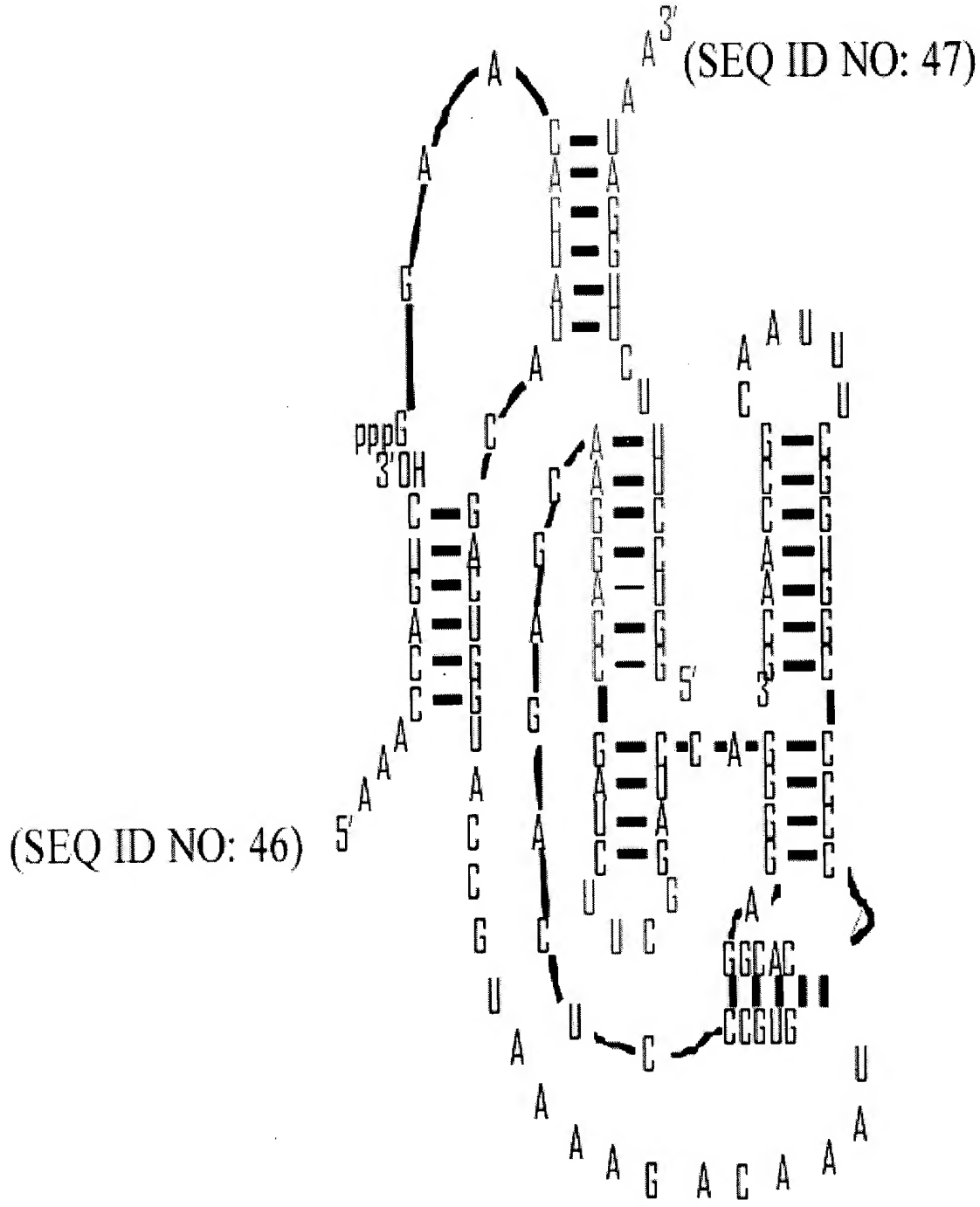


Figure 34: Secondary structure of HCV 5'-UTR